CHARACTERIZING MOISTURE DAMAGED BUILDINGS – ENVIRONMENTAL AND BIOLOGICAL MONITORING

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ACADEMIC DISSERTATION

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ABSTRACT

Moisture and mold problems in buildings are known to cause health effects, but the causal agents of the exposure and the mechanisms of the health effects are obscure. To understand this phenomenon it is important to know how the indoor environment of a moisture damaged building differs from that of a normal, non-damaged building. In this thesis, the differences between moisture damaged buildings and reference buildings in indoor air pollutants, especially microbes, were studied in 38 residences, six day-care centers and two schools with several methods including microbial sampling of air, surfaces, building materials and dust, determination of volatile organic compounds, formaldehyde and house dust mites. In addition, microbial diversity and concentrations in different building materials with moisture damage were characterized. Furthermore, the associations between moisture and mold damage in building and symptoms or mold-specific IgG levels of occupants were examined.

The wintertime concentrations of total viable fungi and concentrations of *Penicillium*, *Aspergillus,* and yeasts in the moisture damaged buildings were higher than in the reference buildings. Higher levels of fungi were observed especially in the particle size fraction of 2-3 µm. In addition, the fungal diversity was larger in the moisture damaged buildings. Certain fungal genera, such as *Stachybotrys, Ulocladium, Tritirachium* and *Exophiala*, were detected only in the air of the moisture problem buildings. No differences were observed in the concentrations or occurrence of the other parameters: airborne viable bacteria, TVOC, formaldehyde, fungi in house dust and house dust mites

The fungal concentrations in moisture damaged and reference buildings overlapped in most cases in moisture damaged and reference buildings, and hence no absolute level could be said to typically indicate the existence of moisture damage. However, by examining both the levels and flora of the air samples, indications of moisture problems can be achieved. The determination of microbial levels and flora especially in building materials, but also on surfaces, were shown to give additional information on the microbial flora in building and this knowledge can be utilized in source characterization.

The temporal variation of the fungal concentrations was significant both in index and reference residences, whereas spatial variation affected mostly the levels in the index residence. In order to reliably ascertain the fungal level of a residence, a sampling campaign of 11 different sampling days in two rooms was proposed.

Fungal diversity in moisture damaged building materials was large. *Stachybotrys* was associated with gypsum boards. *Acremonium, Aspergillus versicolor* and actinobacteria were associated with ceramic materials and they occurred often together on the other materials as well. Sphaeropsidales and yeasts occurred often concurrently in damaged building materials.

Mold-specific serum IgG levels were associated with only a few microbial findings. The occurrence of elevated serum levels was contradictory in exposed and non-exposed populations in different studies. It can be concluded that mold-specific serum IgG levels are not sensitive enough to indicate the current exposure in a moisture damaged home or school environment.

To Ari, Toni, Mika Matti and Essi

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Anne Hyvärinen

ABBREVIATIONS

AIHA	American Industrial Hygiene Association
AM	arithmetic mean
a_w	water activity
BHR	bronchial hyperreactivity
CFU	colony forming unit
CI	confidence interval
CMA	corn meal agar
d_{50}	cut off size
DG18	dichloran 18% glyserol agar
EIA	enzyme immunoassay
ELISA	enzyme –linked immunosorbent assay
EPS	extracellular polysaccharides
EU	endotoxin unit
GC-MS	gas chromatography-mass spectrometry
GM	geometric mean
HCHO	formaldehyde
HDM	house dust mite
HPLC	high pressure liquid chromatography
HVAC	heating ventilating and air-conditioning
IEH	Institute of Environment and Health
IgE	immunoglobulin E
IgG	immunoglobulin G
IND	index
I/O-ratio	indoor/outdoor –ratio
LAL	*Limulus* amebocyte lysate assay
LPS	lipopolysaccharide
MD	median
MEA	malt extract agar
MVOC	microbial volatile organic compounds
NIOSH	National Institute of Occupational Safety and Health
ODTS	organic dust toxic syndrome
OR	odds ratio
PBS	phosphate buffered saline
PCR	polymerase chain reaction
PDA	potato dextrose agar
REF	reference
RH	relative humidity
SBS	sick building syndrome
TLC	thin layer chromatography
TVOC	total volatile organic compounds
TYG	tryptone yeast glucose agar
VOC	volatile organic compounds
WAGM	weighted average geometric mean
YMA	yeast and mold agar

LIST OF ORIGINAL PUBLICATIONS

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II Hyvärinen A, Reponen T, Husman T, Nevalainen A. Comparison of the indoor air quality in mold problem and reference buildings in subartic climate. Central European Journal of Public Health 2001, 9(3):133-139.

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1 INTRODUCTION

Moisture and mold problems in buildings are associated with health effects of both adults and children occupying these buildings (Verhoeff and Burge 1997, Peat *et al.* 1998). Moisture problems can be found in all kinds of buildings; they mostly occur as a consequence of leaks in roofs or in plumbing or capillary movement of water in the structures, but also condensation may take place in cases of poor ventilation or insufficient insulation (Nevalainen *et al.* 1998, Haverinen *et al.* 1999). Moisture problems are common in the modern building stock; approximately 55% of Finnish residences have been estimated to be in need of repair or more thorough inspection due to moisture faults (Nevalainen *et al.* 1998). The costs to health care attributable to the moisture damage in residences have been estimated to be high, high enough to impact on the national economy (Nguyen *et al.* 1998).

Moisture and associated health problems in building are a complex phenomenon involving several complicated and inter-related components. The understanding of moisture damage in buildings requires knowledge of building structures and moisture physics. Excessive moisture in buildings can cause microbial deterioration of the materials and microbial exposure to the occupants adding another factor to the equation. The detrimental consequence of moisture damage, the symptoms and diseases of people occupying the damaged building, demand for medical and epidemiological expertise. One of the underlying problems in this complex entity is that the causal agents of the exposure and the mechanisms of the health effects are not known (Bornehag *et al.* 2001). The exposure in moisture damaged buildings has been described with several methods showing several potential exposing agents. Because the causal relationships are still obscure, all these methods are probably only surrogates of actual exposure. To understand and prevent this complex phenomenon one has to ascertain, how the indoor environment of a moisture damaged building differs from that of a normal, non-damaged building. In addition, there is a demand to gain information for practical situations, how to detect moisture damage and how to reveal health outcomes and prevent them to occur.

2 REVIEW OF LITERATURE

2.1 Moisture damage and health

Indoor air complaints are one of the most common environmental health problems. A great many complaints are connected with moisture problems and microbial growth in buildings, a phenomenon having several names depending on its appearance and severity, such as dampness, damp, damp or moisture patches, water damage, moisture damage, condensation, visible mold, mold growth or fungal growth. The association between health and various moisture problem indicators has been studied extensively, in more than 100 studies as reviewed by Verhoeff and Burge (1997), Peat *et al.* (1998) and Bornehag *et al.* (2001).

The adverse health effects associated with dampness and mold appear mainly as respiratory symptoms (cough, wheeze and asthma), but also as unspecific symptoms like tiredness and headaches (Peat *et al.* 1998, Bornehag *et al.* 2001). The health findings are quite similar in various climatic areas and among both children and adults. Irritative symptoms in the eyes, respiratory tract and skin have been observed in the occupants of moldy buildings, and in some cases, exposed people even develop outright allergies (Waegemakers *et al.* 1989, Husman 1996, Garrett *et al.* 1998, Reijula 1998). In addition, the prevalence of respiratory infections has been abnormally high in occupants of moldy buildings (Waegemakers *et al.* 1989, Brunekreef *et al.* 1989, Pirhonen *et al.* 1996, Husman 1996, Koskinen 1999). Alveolitis, organic dust toxic syndrome (ODTS) and other chronic pulmonary diseases may also develop, although they are usually associated with high exposure in occupational environments such as agriculture (Lacey and Crook 1988, Husman 1996, Reijula 1998).

The association between health effects and building-related moisture and mold is well demonstrated, but the causative agents and mechanisms that lead to the symptoms remain poorly known (Bornehag *et al.* 2001). Sensitization to mite allergens can explain only part of the adverse health effects encountered in moldy buildings (Bornehag *et al.* 2001). Even though the mechanisms and causal factors are still obscure, extensive moisture damage and fungal growth in buildings are unacceptable and should be

removed and prevented (AIHA 1996, Flannigan *et al.* 1996, Ministry of Social Affairs and Health 1997). One important challenge in this research field is to reveal the causal relationships behind the complex phenomenon.

2.2 Exposing agents in moisture damaged buildings

Moisture damage is an event where undesired accumulation of water takes place, thus moistening structural components, insulation materials or the surface material of the building. Moisture damage may be a consequence of leaks in the roof or the plumbing, due to condensation in cases of poor ventilation or insufficient insulation or due to capillary movement of water from soil. Regular or extensive moistening of materials promotes to various chemical and microbial deterioration processes, which may lead to release of chemical and biological emissions into indoor air. However, the relative importance of these various agents as possible causative factors to health effects remains obscure.

Biological particles represent one type of the possible exposing agents with health significance in the indoor air of moisture problem buildings. These particles consist of a variety of fungal, bacterial and other biological materials. In addition to microbial spores and cells, several structural components of microbes, such as ergosterol, the principal sterol in membranes of hyphae and spores of fungi; endotoxin, the cell wall component of gram-negative bacteria; or β(1→3)-glucans, glucose polymers originating from fungi and yeasts and some bacteria, may have health relevance or they can be, at least, used as exposure indicators. Microbial growth may also produce volatile organic compounds (VOCs) and microbial toxins in their metabolism (Sunesson *et al.* 1996, Korpi *et al.* 1998, Pohland 1993). High levels of indoor humidity can also increase the populations of house dust mites present in homes (Konsgaard 1983).

In addition to microbiological deterioration, increased moisture content in materials may enhance emissions due to chemical breakdown (Batterman 1995). In particular, the emissions of formaldehyde depend on moisture conditions in the building (Reponen *et al.* 1991, Tucker 1991). All of these moisture associated indoor air pollutants may all contribute to the symptoms of occupants (Jones 1999).

2.3 Sources of microbes in buildings

2.3.1 Outdoor air

Outdoor air is usually the main source for airborne fungi in indoor environments (Gravesen *et al.* 1979, Burge 1990, Levetin 1995). The fungal population in outdoor air is diverse, but consists mainly of phylloplane mycoflora, such as *Cladosporium, Alternaria, Aureobasidium* together with *Penicillium*, *Aspergillus, Botrytis* and groups of fungi such as yeasts and basidiomycetes (Tobin *et al.* 1987, Li and Kendrick 1995b, Beaumont *et al.* 1985, Cooley *et al.* 1998). The concentrations of viable fungi vary typically from 10^2 to 10^4 cfu/m^3 in summer conditions, while the levels are lower in winter, generally <10^2 cfu/m^3 (Macher *et al.* 1991, Reponen *et al.* 1992, Kuo and Li 1994, Li and Kuo 1994). Geographical and climatic factors play a major role in airborne fungal concentrations. In cold climates with snow cover on the ground, the seasonal variation is great, and in winter, outdoor concentrations of microbes are extremely low due to the snow cover and thus these have only little influence on the indoor air mycoflora (Reponen *et al.* 1992). In warmer climates and also in a cold climate during the frost-free seasons, the concentrations of indoor air fungi follow mainly outdoor concentrations (Fradkin *et al.* 1987, Li and Kuo 1994, DeKoster and Thorne 1995, Dharmage *et al.* 1999b, Burge *et al.* 2000, Su *et al.* 2001). This impact of outdoor air has normally been estimated by using indoor/outdoor ratios of viable fungi or specific genera (Fradkin *et al.* 1987, Kuo and Li 1994, DeKoster and Thorne 1995, Burge *et al.* 2000, Su *et al.* 2001). Even in frost-free seasons, there is a considerable temporal variation in fungal concentrations (Burge 1990).

The transport of spores from outdoor air into indoor air is influenced by the type of ventilation. Mechanical ventilation systems with a filtration of the supply air have been observed to reduce indoor air concentrations of fungi (Reponen *et al.* 1989, Reponen *et al.* 1992, DeKoster and Thorne 1995, Parat *et al.* 1997, Burge *et al.* 2000). Microbes can also enter the building through open doors and windows as well as via uncontrolled air streams through the structures, although the building frame can act as a filter (Thatcher and Layton 1995).

2.3.2 Normal sources

Everyday activities, such as handling foodstuffs or firewood, cleaning and other household activity may release microbes into the indoor air causing occasionally even high concentrations (Hunter *et al.* 1988, Lehtonen *et al.* 1993). While frequent vacuuming has been demonstrated to decrease indoor air concentrations (Dharmage *et al.* 1999b), vacuuming of contaminated carpet may increase airborne concentrations (Buttner *et al.* 2001). Pets and their bedding materials increase the concentrations of viable fungi in air or β(1→3)-glucans in dust (Lehtonen *et al.* 1993, DeKoster and Thorne 1995, Dharmage *et al.* 1999b, Gehring *et al.* 2001, Ren *et al.* 2001). In addition, microbes can be carried indoors on the clothes of people after their work or visits to highly contaminated environments such as barns or stables (Pasanen *et al.* 1989). Spores may also drift through air movements within a building or between buildings especially if they are connected with corridors (Rautiala *et al.* 1996, Morey 1993). Storage of separate organic household waste indoors can be a significant source of microbial contamination of homes (Wouters *et al.* 2000). Other housing characteristics also affect microbial levels; elevated β(1→3)-glucan and fungal levels in house dust or indoor air have been associated with wall-to-wall carpets or plants (Li and Kendrick 1995a, Wouters *et al.* 2000, Gehring *et al.* 2001, Pessi *et al.* 2002). Fungi can also be transported into the indoor air from a crawl-space type of basement with a dirt floor and basements or cellars in general (Su *et al.* 1992, Lehtonen *et al.* 1993, DeKoster and Thorne 1995, Ren *et al.* 1999).

House dust has been considered as a reservoir of fungi, from which spores can be resuspended into the air. The importance of this phenomenon can, however, be questioned, since of the several fungal species found in house dust, only half of them were detected in indoor air (Miller *et al.* 1988). In normal dry conditions, fungal spores do not germinate or grow in the indoor environment, but are removed by gravitational settling or by ventilation. However, spores can develop into microcolonies on surfaces that occasionally become wet (Pasanen *et al.* 1992a).

Fungal spores gradually accumulate in buildings. Thus, indoor air concentrations of viable fungi are higher in older than new residences (Pasanen 1992). This also appears

as high concentrations of airborne fungi during demolition of building structures (Rautiala *et al.* 1996).

The indoor air of occupied buildings typically has higher levels and a different flora of bacteria than outdoor air (Nevalainen 1989, Otten and Burge 1999). The main source for indoor air bacteria is humans (Nevalainen 1989). The levels of bacteria in Finnish buildings increased significantly after the occupants had moved in (Reponen *et al.* 1989). However, no such difference in pre- and post-occupancy periods were found in microbial concentrations in a two year sampling period in San Francisco, CA (Macher *et al.* 1991).

2.3.3 Microbial growth

Dampness and moisture

Microbes are ubiquitous in building structures, and microbial growth starts when the moisture conditions permit. Thus, moist building materials provide a potential and unwanted source of microbes. The source strength of microbial contamination depends on type, seriousness, extent, age and location of the moisture damage. In fact, due to the strong association between such microbial growth and health effects (see section 2.1), microbial growth can be regarded as one of the most important causes of indoor air problems and complaints. Visible mold or dampness in buildings are associated with elevated concentrations of fungi in air (Hunter *et al.* 1988, Li and Kendrick 1995a, Garrett *et al.* 1998, Dharmage *et al.* 1999b, Ellringer *et al* 2000) and in house dust (Verhoeff *et al.* 1994b) as well as with an increased ergosterol content in dust (Dharmage *et al.* 1999b). Microbial growth inside insulated external walls has been noted to increase the levels of the actinobacteria in the indoor air, but not the levels of fungi (Pessi *et al.* 2002). The demolition of moldy structures has been observed to create very high airborne fungal levels (Rautiala *et al.* 1996).

The ducts of heating, ventilating and air-conditioning (HVAC) systems can also act as sources of fungal contamination (Bernstein *et al.* 1983, Garrison *et al.* 1993). Water condensation may take place in ducts, which are often located in unheated areas of a building. Also uncovered insulation material within ducts may support microbial growth

(Foarde *et al.* 1997). In the case where the growth takes place in the ducts of supply air, the ventilation system acts as a distribution mechanism for the spores into indoor air (Rossi *et al.* 1991). This type of source can be eliminated by the sanitation of air handing systems (Garrison *et al.* 1993, Levetin *et al.* 2001) or increased efficiency of filtration.

Factors affecting microbial growth

The most important factors that affect microbial growth on building materials are moisture, nutrients, and temperature. In principle, water availability, usually discussed in terms of water activity (a_w), is the most critical factor for microbial growth. Moisture demands depend on fungal genus or species (Grant *et al* 1989), but, in general, most mesophilic molds can grow at a_w of 0.95-0.99, while the range for xerophilic molds and for yeasts are 0.65-0.90 and 0.88-0.99, respectively (Gravesen *et al.* 1994). On materials with limited nutrients, the minimal a_w is higher as is the case with lower than optimum temperatures (Grant *et al.* 1989, Flannigan 1992a). Fungal growth can start rapidly when moisture conditions are optimal in the material (Adan 1994). Rapid drying of a material decreases the viability of the spores (Pasanen *et al.* 2000b), even though some fungi (e.g. *Penicillium)* can tolerate fluctuating moisture conditions (Adan 1994, Korpi *et al.* 1998). Relative humidity (RH) of air has no direct influence on fungal growth, because fungi can grow at very low air humidities if there is enough moisture on the surface (Gravesen *et al.* 1994). Condensation on cold surfaces, however, is possible and the humidity of air can influence the moisture content of material; an RH value of 70% is often considered to be critical (Hunter *et al.* 1988, Grant *et al.* 1989). No colonization of fiberglass insulation was seen at RH below 50% (Ezounu *et al.* 1994).

Fungi and bacteria need external sources of nutrition: carbon, nitrogen, phosphorous, and potassium (Dix and Webster 1994), which are usually provided by most building materials. Trace amounts of nutrients occur even in house dust and water and therefore, the availability of nutrients does not generally limit the microbial growth. Fungi can indeed grow on materials such as fiberglass and on galvanized steel which has an accumulated dust layer or a residue of lubricant oil (Pasanen *et al.* 1993a, Pasanen *et al.* 1993b, Chang *et al.* 1996, Buttner *et al.* 1999). The temperature in buildings, typically 20-25°C, promotes mainly mesophilic microbes, that have their optimal growth

temperature 20-<45°C (Ingold and Hudson 1993, Atlas and Bartha 1993). However, microbes may grow slowly at temperatures well below their optimal (Atlas and Bartha 1993, Hyvärinen *et al.* 1991). In addition, thermophilic microbes growing at 35-90°C are occasionally found in buildings (Burge and Otten 1999). Most of the fungi grow best in rather neutral circumstances, optimally in the pH range 5-6.5 (Ingold and Hudson 1993), which is also the pH range of most building materials. Because there is usually also enough light and oxygen available in the buildings, the availability of water remains the critical factor regulating the possible growth of microbes in indoor environments.

2.4 Behavior of microbes in indoor environments

Different fungal genera have different capacities to release spores, which partly explains the fungal composition of indoor air. For example, dry spores of *Penicillium* and *Aspergillus* are more easily released into air than *Cladosporium* spores under the same indoor air conditions (Pasanen *et al.* 1991). *Penicillium commune* more easily releases spores than *Aspergillus versicolor* or *Paecilomyces variotii* (Näsman *et al.* 1999). This may partly explain the frequent occurrence of *Penicillium* in indoor air (Hunter *et al.* 1988, Miller *et al.* 1988, Pasanen 1992, Kuo and Li 1994, Beguin and Nolard 1994, Ren *et al.* 1999, Górny *et al.* 1999, Burge *et al.* 2000). Fungi producing spores in slime, such as *Stachybotrys* and *Acremonium,* or those producing spores in closed fruiting bodies, such as *Chaetomium*, are not able to release spores easily (Samson *et al.* 1996), thus the finding of their spores in air is less probable.

The release of spores of fungi and also sporulating bacteria such as actinobacteria are affected by external factors such as air humidity (Pasanen *et al.* 1991, Reponen *et al.* 1998, Foarde *et al.* 1999) and air velocity (Zoberi 1961, Pasanen *et al.* 1991, Foarde *et al.*1999, Górny *et al.* 2001), as well as the texture of the surface and vibration of contaminated material (Górny *et al.* 2001). More spores were released at a higher air velocity and at lower air humidity, while air turbulence increased the release from a rough surface (Górny *et al.* 2001). A mechanical disturbance of mold growth, such as dismantling, increased the airborne concentration of spores (Hunter *et al.* 1988, Rautiala *et al.* 1996). In the study of Pessi *et al.* (2002), actinobacteria growing in the insulation layer of an external wall were observed to infiltrate to the indoor air, while

fungal contamination originating from the envelope of precast concrete panel buildings was rare.

The behavior of airborne microbes, like particles in general, is strongly affected by the size of the spore or the cell (Reponen *et al.* 2001). All mechanisms for settling, resuspension and removal of microbes are at least partly influenced by the size of the particle. The smaller particles stay airborne longer, whereas the larger particles settle faster. Spores and cells are removed from indoor air by gravitational settling or by air movements due to ventilation. Even though lower concentrations of fungi have been reported in buildings with mechanical ventilation (Harrison *et al.* 1992), the operation of the mechanical ventilation does not always correlate with the airborne concentrations of viable fungi and bacteria (Lawton *et al.* 1998, Lappalainen *et al.* 2001). Settled spores can be resuspended into the air by human activity. Normal walking on a carpet can significantly increase spore counts (Buttner and Stetzenbach 1993). In addition, the higher airborne fungal levels found in schools with carpeted floors than in respective offices were suggested to be due to the higher activity levels in schools (Gravesen *et al.* 1986). Human activity is also known to cause the phenomenon called the “personal cloud” by being close to sources, such as cooking and vacuuming, and by resuspension of coarse particles (Wallace 1996).

The concentrations of viable fungi in indoor air are suggested to have large temporal and spatial variations (Hunter *et al.* 1988, Verhoeff *et al.* 1990, Pasanen *et al.* 1992c). The temporal variation is partly due to the seasonal variation in the fungal concentrations of the outdoor air (Kuo and Li 1994, Reponen *et al.* 1992, Ren *et al.* 1999) and partly due to the activity of the occupants; the highest concentrations are seen during the most intense activity (Flannigan 1992b). The spatial variation of total spores within the same building can be remarkable (Li and Kendrich 1995a), but there are also studies reporting no or little spatial variation between the different rooms of the same building (Dotterud *et al.* 1995, Ren *et al.* 1999, Ross *et al.* 2000, Ren *et al.* 2001). Higher concentrations of fungal spores in the living room have been explained by higher activity there than in other rooms, but also by the influence of outdoor air, because the difference was seen in the concentrations of *Cladosporium* and *Alternaria* (Li and Kendrich 1995a). Spatial variation may also be caused by differences in fungal sources, as the variation seems to be greatest in damp residences, and between living

areas and basements (Pasanen *et al.* 1992c, DeKoster and Thorne 1995, Ren *et al.* 1999). The largest fungal diversity found in the kitchens is thought to reflect the release of spores during food preparation and cooking (Li and Kendrich 1995a).

2.5 Determination of microbes in indoor environments

Determination of concentrations and flora microbes in a building is usually done either in order to estimate the microbial exposure of the occupants or in order to find abnormal sources of microbes. In both exposure assessment and source characterization, it is essential to know what kind of exposure or contamination can be considered as normal or abnormal.

2.5.1 Sampling of bioaerosols

Several methods are available for sampling of bioaerosols. No single sampling method can be used to collect, identify and quantify all bioaerosols at the same time (Nevalainen *et al.* 1992, Reponen *et al.* 2001). The selection of the method must be based on the aim of the measurement, the environment and the resources available. In the sampling of bioaerosols, different physical forces are applied: inertial impaction, centrifugal impaction, liquid impingement, filtration and gravitational settling (Willeke and Macher 1999, Reponen *et al.* 2001).

In inertial impaction, the inertia of the particle forces it to impact on the collection surface. This principle is used in multi-hole impactors, such as one-, two and 6-stage Andersen impactors, Burkard spore trap and surface-air-sampler (SAS) as well as in slit samplers (e.g. agar slide impactor, glass slide impactor). Centrifugal impaction is also based on the inertia of the particle, but in a radial manner. Examples of use of this type of impaction are Reuter Centrifugal samplers (RCS, RCS+) and cyclone samplers (Willeke and Macher 1999). In liquid impingement, particles are mainly collected with inertial impaction, but also due to the diffusion within bubbles (Reponen *et al.* 2001). This principle is employed in all-glass impingers (e.g. AGI-4, AGI-30) and in the BioSampler. The sampling principle of the BioSampler is based on both inertial impaction and centrifugation (Lin *et al.* 1999). In filtration, several forces are used to separate particles from air: inertia, interception, gravitational settling, diffusion and

electrostatic attraction (Willeke and Macher 1999). Examples of filter sampling devices are cassette filters and Button sampler (Willeke and Macher 1999, Aizenberg *et al.* 2000). In gravitational settling, the particles settle passively on the collection surface, which can be an open petri dish supplied with growth medium.

The samplers differ in cut-off size (d_{50}), which is the particle size above which 50% or more of the particles are collected. Impactors have steep cut-off characteristics; therefore in most impactors, d_{50} is assumed to be the size above which all particles larger than that size are collected (Nevalainen *et al.* 1992). There are several studies that have characterized the performance of various samplers (Nevalainen *et al.* 1992, Grinshpun *et al.* 1994, Aizenberg *et al.* 2000) or compared their behavior in field or experimental studies (Heikkilä *et al.* 1988, Smid *et al.* 1989, Verhoeff *et al.* 1990, Verhoeff *et al.* 1992, Jensen *et al.* 1992, Thorne *et al.* 1992, Buttner and Stetzenbach 1993, Juozaitis *et al.* 1994, Cage *et al.* 1996, Mehta *et al.* 1992, Gao *et al.* 1997, Hauck *et al.* 1997, Pahl *et al.* 1997, Lin *et al.* 1999, Bellin *et al.* 2001). The principle of operation, cut-off sizes and application possibilities of commonly used samplers are described in detail by Willeke and Macher (1999).

2.5.2 Analysis of bioaerosols

The analysis of bioaerosol samples is based on either cultivation of the collected microbes into colonies, direct microscopical counting of spores or cells, analysis of chemical markers of biomass, immunochemical analysis of allergens, or determination of biological activity. Recently, PCR-assays for indoor fungi have been developed, which may soon change the routine protocol for microbiological methodology of indoor environment (Haugland *et al.* 1999, Zhou *et al.* 2000, Buttner *et al.* 2001, Cruz-Perez *et al.* 2001a and b, Williams *et al.* 2001).

Cultivation

The determination of airborne levels of fungi and bacteria has been traditionally based on cultivation methods after sampling with impactors directly on growth media (Hunter *et al.* 1988, Miller *et al.* 1988, Reponen *et al.* 1989, Verhoeff *et al.* 1990, Nevalainen *et al.* 1991, Verhoeff *et al.* 1992, Pasanen *et al.* 1992c, Pasanen 1992, Reponen *et al.*

1992, Beguin and Nolard 1994, Lehtonen *et al.* 1993, DeKoster and Thorne 1995, Burge *et al.* 2000, Miller *et al.* 2000). The most widely used sampling device is the Andersen 6-stage impactor, which collects airborne microbes effectively (Willeke and Macher 1999) and allows identification of air spora and examination of microbes in different size classes. The six-stage impactor is, however, suitable only for short periods of air sampling, <20 min. Cultivation has also been made from filter samples (Palmgren *et al.* 1986, Björnsson *et al.* 1995, Rautiala *et al.* 1996) and impinger samples (Jensen *et al.* 1992, Thorne *et al.* 1992, Lin *et al.* 1999). The airborne microbial flora has been determined with gravitational settling samples in a few studies (Rogers 1984, Verhoeff *et al.* 1992, Cvetnic and Pepeljnkak 2001). Even though levels given by this method may correlate with levels given by the 6-stage impactor (Verhoeff *et al.* 1992), the method is semiquantitative at best, because it greatly favors larger particles and is affected by air movements (Reponen *et al.* 2001).

The methods based on cultivation always involve the use of growth media, but no single medium is capable of detecting all of the microbes present in the air. The media that are generally used for detecting fungi in indoor environments include 2% malt extract agar (MEA) for hydrophilic fungi, dichloran 18% glycerol agar (DG18) (Samson *et al.* 1994, AIHA1996) and malt-salt agar for xerophilic fungi and cellulose agar for *Stachybotrys chartarum* (AIHA1996). The choice of the medium influences how readily various genera are detected. For example, MEA favors fast growing *Penicillium* and *Aspergillus* sp. at the expense of slower-growing genera like *Stachybotrys* and *Chaetomium* (Samson *et al.* 1994, Andersen and Nissen 2000), which would be best detected with corn meal agar (CMA) (Tsai *et al.*1999) or V8 juice agar (Andersen and Nissen 2000).

Microscopic counting

Only some of the microbes in indoor air are viable (Näsman *et al.* 1999, Toivola *et al.* 2002) or able to grow on the culture media provided (Burge and Otten 1999). The total number of microbes can be determined with microscopical counting of spores and cells with light microscopy, epifluorescence microscopy or scanning electron microscopy (Palmgren *et al.* 1986, Eduard *et al.* 1990). The preparation of the sample depends on the type of microscope to be used (Heikkilä *et al.* 1988, Eduard *et al.* 1990). When

using the epifluorescence microscope, the counting can also be done with image processing (Kildesø and Nielsen 1997).

Cultivation methods underestimate the total microbial levels, but it has been shown that the viable and total spore concentrations in indoor environments correlate well (Palmgren *et al.* 1986, DeKoster and Thorne 1995). Several estimates have been presented for the proportion of viable/culturable microbes out of the total numbers of airborne biological particles. In dwellings, the number of viable fungi has been observed to be only about 1% of the total counts of the spores (Toivola *et al.* 2002). In agricultural environments, a large variability in this proportion has been observed; Heikkilä *et al.* (1988) and Hanhela *et al.* (1995) have reported 1-25% of fungal spores to be viable, whereas according to Eduard *et al.* (1990) 0.1 – 68% of bacteria and actinobacteria and 3-98% of fungi were viable.

2.6 Microbial concentrations and flora in indoor air

2.6.1 Fungal concentrations and flora

A summary of the airborne levels of fungi in residences is presented in Table 1, in which the studies have been divided into three groups according to the study design: residences with suspected mold damage or other indoor complaints, residences without any known indoor air problems and a few examples of other environments. The designs, sampling methods and results of fungal measurements as well as main conclusions of the studies are shown. The studies are listed in the order of their year of publication.

Table 1 shows that the concentrations of viable airborne fungi vary between 10^1-10^5 cfu/m^3. This wide range is partly explained by the impact of outdoor air (see section 2.3.1). Mean levels are, however, typically 10^2-10^3 cfu/m^3. In two studies, lower indoor levels have been reported in winter; this was noted not only in a cold climate (Reponen *et al.* 1992) but also in a subtropical climate (Kuo and Li 1994). In most studies, the sampling period covered several seasons. The impact of the seasonal variation has either not been taken into account in the studies of indoor fungi or has been resolved by calculating indoor/outdoor ratios of total fungi or genera. The total concentrations of

fungal spores in homes varied between 10^3 – $6*10^5$ spores/m^3 (Björnsson *et al.* 1995, Rautiala *et al.* 1996, Toivola *et al.* 2002). As examples of other environments, fungal levels in a few studies concerning offices and a hotel are shown. The levels in those environments varied between 10-10^4 cfu/m^3 (Table 1).

The most frequently found genus in indoor air has been *Penicillium* together with *Cladosporium, Aspergillus* (Hunter *et al.* 1988, Miller *et al.* 1988, Waegemaekers *et al.* 1989, Strachan *et al.* 1990, Pasanen 1992, Pasanen *et al.* 1992c, Kuo and Li 1994, Li and Kuo 1994, Beguin and Nolard 1994, Dotterud *et al.* 1995, Ren *et al.* 1999, Górny *et al.* 1999, Burge *et al.* 2000) and yeasts (Hunter *et al.* 1988, Pasanen 1992, Pasanen *et al.* 1992c). These common genera and groups are mostly the same, independent of the climate or continent, because the studies originate from Great Britain, Canada, the Netherlands, Finland, Taiwan, Belgium, Norway, USA, and Poland. In several studies, *Cladosporium* has been the most dominating genus and its main source has been outdoor air (Verhoeff *et al.* 1992, DeKoster and Thorne 1995, Dharmage *et al.* 1999b, Ren *et al.* 1999, Su *et al.* 2001). In addition to the most common genera or groups, also other genera e.g. *Ulocladium, Geomyces, Sistotrema* and *Wallemia* have been found relatively often (Hunter *et al.* 1988, Verhoeff *et al.* 1992).

2.6.2 Fungal concentrations in relation to building dampness or moisture

In some studies, the association between elevated fungal levels and moisture damage or observed mold growth has been investigated. There are also a number of reports that present studies of indoor air fungi with disease-based design. Because the overall picture of the complex triangle between building damage, health effects and microbial exposure is unclear, a variety of studies and their main conclusions are summarized in Tables 1 and 2.

The observations of concentrations of viable fungi in moisture damaged residences have been contradictory (Table 1; residences with suspected mold damage or other indoor complaints). In general, fungal concentrations have been higher in moisture damaged buildings than in buildings without such problems (Gallup *et al.* 1987, Waegemakers *et al.* 1989, Verhoeff *et al.* 1992, Li and Kendrick 1995b, Dharmage *et al.* 1999b, Johanning *et al.* 1999, Klánová 2000). Hunter *et al.* (1988) also showed that

there were higher levels of fungi in a room with visible growth than in those rooms where mold was absent. On the other hand, there are many studies where no difference in concentrations of viable fungi between moldy and non-moldy buildings has been observed (Strachan *et al.* 1990, Nevalainen *et al.* 1991, Pasanen *et al.* 1992c, Pasanen 1992, Dill and Niggemann 1996, Garrett *et al.* 1998) or between homes with severe and mild mold damage (Miller *et al.* 2000). Furthermore, fungal growth in the insulated external wall of precast concrete panel buildings has not been found to affect the indoor air levels (Pessi *et al.* 2002). In some studies reporting fungal levels, the residences have been defined as complaint buildings with no description on moisture-related indoor air problem (Table 1). In these studies, higher concentrations of fungi or I/O-ratio of the fungal concentration have indicated indoor air sources for fungi (Reynolds *et al.* 1990, DeKoster and Thorne 1995). In addition, fungal levels have been observed to increase during the demolition of moldy structures or constructional work (Hunter *et al.* 1988, Rautiala *et al.* 1996), but decrease back to baseline level in a few months after removal of the damaged materials (Rautiala *et al.* 1996, Ellringer *et al.* 2000).

In most studies, the classification of residences is based on reported or observed visible mold. There are only a few studies that have investigated levels in buildings with no moisture or mold damage. The range or average of the fungal concentrations in residences with or without mold or moisture damages have not been always reported, which makes the comparison difficult. In general, the distributions of fungal levels in moldy and non-moldy buildings overlap. Extremely high levels (e.g. 23 000 cfu/m^3) have been reported even in residences with no visible mold in a study that showed the association between mold damage and fungal levels (Hunter *et al.* 1988). Only in the studies of Klánová (2000) and Johanning *et al.* (1999) was the difference in ranges of fungal levels fairly clear. Based on these studies, there is no fungal level that always indicates moisture or mold damage, even though several attempts to set such limits have been reported (Rao *et al.* 1996). In order to use fungal levels in source characterization, the conclusion must be based on the knowledge of what is considered normal in the environment and climate of interest.

In Table 1, several studies are also listed that deal with residences without any known indoor air problem, in order to describe the overall fungal levels of residences. The

levels vary between 10 –10^5 cfu/m^3 and thus overlap with the levels observed in the residences with moisture or mold problem. These studies have examined several factors accounting for the variation in fungal levels, such as seasonal variation, outdoor air and ventilation (see also sections 2.3.1 and 2.3.2).

Table 1. The summary of the airborne fungal levels in residences and a few other environments and the relation between levels and building characteristics or outdoor air.

STUDY	NUMBER AND TYPE OF SITES	STUDY DESIGN	METHOD	LEVELS OF AIRBORNE FUNGI	FUNGAL LEVELS IN RELATION TO BUILDING CHARACTERISTICS
Residences with suspected mold damage or other indoor complaints					
Gallup *et al.* 1987	127 residences	Moisture problem Non-problem	6-stage impactor	problem : AM 5950 cfu/m^3 non-problem: AM 716 cfu/m^3	Levels in problem homes higher
Hunter *et al.* 1988	62 residences	Monitoring complaint homes	6-stage impactor (MEA)	Visible mold: <12-449 800 cfu/m^3 No mold: <12- 23 070 cfu/m^3	High levels of fungi associated with visible mold growth, constructional work and activity
Miller *et al.* 1988	50 residences	Characterize levels of fungi and fungal metabolites in winter	RCS (Rose bengal malt extract) Filter (Rose bengal malt extract, MEA + sucroce)	RCS : AM 345 cfu/m^3 (0-3125) Filter : AM 111 cfu/m^3	No conclusion on effect on moisture or dampness
Waegemaekers *et al.* 1989	36 residences	Damp (24) Reference (8)	6-stage impactor (MEA)	Damp: GM 192 cfu/m^3 Reference: GM 102 cfu/m^3	Fungal levels associated with dampness.
Strachan *et al.* 1990	88 residences	Homes of children with wheeze (34) and Controls (54)	6-stage impactor (MEA)	Visible mold: <41 300 cfu/m^3 (MD 200-294) No mold: < 38 600 cfu/m^3 (MD 21-283)	Median concentrations of viable fungi did not associate with visible mold.
Reynolds *et al.*1990	6 residential and office environments	Monitoring complaint buildings	2-stage impactor (Sabouraud dextrose agar)	Indoors: <18900 cfu/m^3 Outdoors: <1090 cfu/m^3	High I/O and flora indicated indoor air sources.
Nevalainen *et al.*1991	48 residences	Mold damaged (30) Reference (18)	6-stage impactor (Hagem)	Damaged: 10-2300 cfu/m^3 (GM 102) Reference: 165-850 cfu/m^3 (GM 308)	The mean concentrations of viable fungi lower in the damaged than reference residences, but higher mean I/O-ratio in the damaged residences (4.2/0.6) indicates indoor sources.
Pasanen *et al.*1992c	46 residences	Damp (25) Reference (21)	6-stage impactor (Hagem)	Damp: <2291 cfu/m^3 (GM 80) Reference: <1445 cfu/m^3 (GM 78)	Levels not higher in damp houses.

Continued

Table 1. continued

STUDY	NUMBER AND TYPE OF SITES	STUDY DESIGN	METHOD	LEVELS OF AIRBORNE FUNGI	FUNGAL LEVELS IN RELATION TO BUILDING CHARACTERISTICS
Pasanen 1992	57 residences	Urban (21) Damp urban (22) Rural (13): 7old+ 6new	6-stage impactor (Hagem/MEA)	Urban: <1445 cfu/m^3 (GM 78) Damp: 2-1198 cfu/m^3 (GM 69) New rural: 25-1916 cfu/m^3 (GM 70) Old rural: 98-5730 cfu/m^3 (GM 1012)	Levels not higher in damp houses. Levels higher in old rural houses.
Verhoeff *et al.* 1992	130 residences	Relation of fungal levels on dampness	1-stage impactor (DG18) Sedimentation (DG18) (in 84 residences)	Indoors: 62-43045 cfu/m^3 (GM 640-822) Sedimentation: 0-518 cfu	Fungal levels correlated weakly with house damp.
Beguin and Nolard 1994	130 residents	Monitoring patient homes	RCS (HS medium with rose bengal)	375-3750 cfu/m^3	No conclusion on effect on moisture or dampness.
DeKoster and Thorne 1995	41 residences:	Health based home categories: Non-complaint (27) Intervention (10) Complaint (4)	6-stage impactor (MEA)	Noncomplaint: GM <1290 cfu/m^3 Intervention: GM <1100 cfu/m^3 Complaint: GM <6700 cfu/m^3	I/O-ratios higher in complaint homes. Levels higher in the basement than main floor.
Li and Kendrick 1995b	15 residences	Homes of allergic (13) Homes of non-allergic (2)	Samplair MK1/MK2	Damp: 2727 spores/m^3 Non-damp: 2051 spores/m^3	Levels higher in the damp residences.
Rautiala *et al.* 1996	7 buildings	Monitoring of effect of mold damage repair	6-stage impactor (MEA) filter	Before repairs: GM 370 cfu/m^3 (<1150) GM 59000 spores/ m^3 (<500000) After repairs: GM 200 cfu/m^3 (<300)	Demolition of moldy structures increase levels remarkably. Levels on baseline after 6 months.
Dill and Niggemann 1996	20 residences	Homes of children with allergic diseases	RCS (MEA+Czapek Dox)	Visible mold: 64 – >4000 cfu/m^3 No mold: <13 – 1652 cfu/m^3	Airborne levels did not correlate with visible fungal growth.

Continued

Table 1. continued

STUDY	NUMBER AND TYPE OF SITES	STUDY DESIGN	METHOD	LEVELS OF AIRBORNE FUNGI	FUNGAL LEVELS IN RELATION TO BUILDING CHARACTERISTICS
Garrett *et al.*1998	80 residences	Homes of asthmatics (43) and non-asthmatic (37)	1-stage impactor (MEA)	<20-54749 cfu/m^3 (MD 812)	No association between mean levels and visible mold. Elevated levels of fungi associated with musty odor, moisture or humidity, poor ventilation and failure to clean indoor mold growth.
Rautiala *et al.* 1998	3 buildings	Reducing microbial exposure during demolition of moldy structures	Filter cultivation (MEA, DG18)	Before repairs: 860-1300 cfu/m^3 During repairs: <8*10^5	Local exhaust method most effective for control. Personal protection still needed.
Dharmage *et al.* 1999b	485 residences	Homes of random sample (349) and asthmatics (139)	2-stage impactor (PDA)	37-7619 cfu/m^3 (MD 549)	Higher concentrations in residences with visible mold.
Johanning *et al.* 1999	2 residences	Mold damaged Control	1-stage impactor (MEA) Filter	Damaged: 1993->7069 cfu/m^3 1.8-6.6 *10^5 spores/m^3 Control: 194-336 cfu/m^3 3.7-4.7 *10^3 spores/m^3	Higher concentrations in residence with visible mold.
Klánová 2000	Residences and offices 68 rooms: ,	A) no complaints + no mold (20) B) complaints + no mold (20) C) no complaints + visible mold (10) D) complaints+ visible mold (18)	RCS Plus aeroscop (YMA)	A) 0 - 230 cfu/m^3 (AM 78) B) 0 - 140 cfu/m^3 (AM 58) C) 60 - 3190 cfu/m^3 (AM 1033) D) 120 - 17930 cfu/m^3 (AM 2476)	Concentrations of viable fungi higher in rooms with visible mold.
Miller *et al.* 2000	58 residences	Relation of air sampling and damaged materials	RCS (Rose bengal malt extract)	15 residences with lowest visible growth: AM 214 cfu/m^3 15 residences with highest visible growth: AM 329 cfu/m^3	The mean levels were not associated with severity of damage. More species different from outdoor air in homes with severe damage.
Pessi *et al.* 2002	88 residences	Fungal levels in relation to the microbial growth in external walls	6-stage impactor (MEA)	Low growth: 9 - 516 cfu/m^3 (AM 112) Growth: 2 - 1784 cfu/m^3 (AM 121)	Microbial growth in insulated external wall did not effect indoor air levels.

Continued

Table 1. continued

STUDY	NUMBER AND TYPE OF SITES	STUDY DESIGN	METHOD	LEVELS OF AIRBORNE FUNGI	FUNGAL LEVELS IN RELATION TO BUILDING CHARACTERISTICS
Residences without any known indoor air problems					
Fradkin *et al.* 1987	27 residences	Species identification	2-stage impactor (Rose bengal agar)	Indoor: AM 742 cfu/m^3 Outdoor: AM 1131 cfu/m^3	Species identification allows for the detection of deviations from outdoor air
Pasanen *et al.* 1989	3 residences	Farm houses (2) Urban residence	6-stage impactor (MEA) filter	Farm house: 10^4-10^5 cfu/m^3 10^5-10^6 spores/m^3 Urban: <100 cfu/m^3 10^4-10^5 spores/m^3	Fungal spores are carried from barns to farmers' homes.
Reponen *et al.* 1989	18 residences	Effect of ventilation and occupancy	6-stage impactor (Hagem)	3-1300 cfu/m^3 (GM 140)	Mechanical supply and exhaust ventilation decreases levels. The first year occupancy has no effect on fungal levels
Macher *et al.* 1991	A new residence	Temporal variation and effect of occupancy	1-stage impactor (MEA)	Indoors: 58-673 cfu/m^3 [a] (MD 198) Outdoors: 113-1158 cfu/m^3 [a] (MD 362)	No change in microbial contamination due to occupancy. Elevated outdoor humidity, lower air temperature and wind associated with higher levels.
Reponen *et al.* 1992	71 residences	Non-complaint Seasonal variation Effect of ventilation	6-stage impactor (rose bengal malt extract)	Indoor, summer: GM 410 cfu/m^3 Indoor, winter: GM 40 cfu/m^3 Outdoor, summer: GM 950 cfu/m^3 Outdoor, winter GM 20 cfu/m^3	Indoor and outdoor levels an order of magnitude lower in winter than summer. Ventilation systems affect levels only in summer: levels are the lowest with mechanical supply and exhaust ventilation system.
Kuo and Li 1994	6 residences	Seasonal variation in subtropical climate	1-stage impactor (MEA)	Indoors: <3202 cfu/m^3 Outdoors: <4200 cfu/m^3 Winter, indoor: <165 cfu/m^3 Winter, outdoor: <203 cfu/m^3	Seasonal variation remarkable: winter levels clearly lower
Li and Kuo 1994	12 residences	Characteristics of microfungi in subtropical climate	2-stage impactor (MEA)	Indoors: 1050-4970 cfu/m^3 Outdoors: 770-5740 cfu/m^3	
Górny *et al.* 1999	60 residences	Size distributions	6-stage impactor (MEA)	<10^4 cfu/m^3	

[a] 95% confidence interval

Continued

Table 1. continued

STUDY	NUMBER AND TYPE OF SITES	STUDY DESIGN	METHOD	LEVELS OF AIRBORNE FUNGI	FUNGAL LEVELS IN RELATION TO BUILDING CHARACTERISTICS
Ren *et al.* 1999	11 residences	Seasonal variation	Burkard (DG18, MEA)	Winter: Indoor: GM 314-432 cfu/m^3 (basement 1658) outdoor: GM 505 cfu/m^3 other seasons: indoor: GM 705-1036 cfu/m^3 (basement 988-1165) outdoor: GM 607-1198 cfu/m^3	Levels higher in summer reflecting outdoor air. The levels in basements higher than in other indoor areas and outdoor in winter. No seasonal variation in basements.
Wu *et al.* 2000b	76 residences	Winter -summer Urban-suburban	Burkard (MEA)	Winter: Indoor: GM 8946 cfu/m^3 Outdoor: GM 11464 cfu/m^3 Summer: Indoor: GM 4381 cfu/m^3 Outdoor: GM 4689 cfu/m^3	In summer, levels higher both indoors and outdoors in suburban homes. I/O-rations of Penicillium and Aspergillus higher in suburban homes.
Ren *et al.* 2001	1000 residences	Relation to house characteristics	Burkard (DG18, MEA)	0-14900 cfu/m^3 (MD 300-450)	Levels related to season, relative humidity, temperature and presence of cat. Presence of fungi in air cannot be predicted from reported home characteristics.
Cvetnic and Pepeljnkak 2001	55 residences	Urban (32) and rural (33) residences	Sedimentation	0-100 cfu/plate/15 min	Fungal colony counts higher in rural homes
Toivola *et al.* 2002	81 residences	Comparison of personal, work and home exposure	Filter: Total + MEA/DG18	Homes: MD 5-6 cfu/m^3 MD 4700 spores/m^3 Work: MD 2-3 cfu/m^3 MD 9000 spores/m^3 Personal: MD 12 cfu/m^3 MD 5700 spores/m^3	Personal exposure higher than home and work exposure.
Other environments					
Harrison *et al.* 1992	15 offices	SBS and airborne microbes	6-stage impactor (MEA)	2-978 cfu/m^3 (MD 26-277)	Levels higher in naturally ventilated buildings.
Morey 1993	3 office buildings	One damaged by fire and moisture Two connected to the damaged building	A single stage sieve (MEA)	40 - >10^4 cfu/m^3	Spores migrated from damaged areas to non-damaged areas. Air sampling indicated hidden damage.

Continued

Table 1. Continued

STUDY	NUMBER AND TYPE OF SITES	STUDY DESIGN	METHOD	LEVELS OF AIRBORNE FUNGI	FUNGAL LEVELS IN RELATION TO BUILDING CHARACTERISTICS
Burge *et al.* 2000	An office building	Follow-up a new building	2-stage impactor (2% MEA, DG18)	Indoor: 10-372 cfu/m^3 Outdoor: 99-3195 cfu/m^3	Air sampling did not indicate growth in ventilation system.
Ellringer *et al.* 2000	A hotel	Mold intervention	Filter and cultivation (2%MEA)	Indoor: 75-2946 cfu/m^3 Outdoor: 151-338 cfu/m^3	Levels decreased to normal with removal of damaged materials and cleaning

2.6.3 Fungal concentrations in relation to health effects

The studies reporting the relationships between airborne fungal indoor levels and health effects are listed in Table 2, which shows the design with the health point of interest, sampling method and the fungal results with the main observation of each study, in the order of the publication year of the study. The elevated concentrations of fungi in residences have been associated with several health effects, such as respiratory symptoms, sensitization to house dust mites, asthmatic symptoms or emergency room visits due to asthma (Waegemaekers *et al.* 1989, Dotterud *et al.* 1995, Björnsson *et al.* 1995, Johanning *et al.* 1999, Ross *et al.* 2000, Dharmage *et al.* 2001, Jarvis and Morey 2001) (Table 2). Associations between elevated fungal levels and respiratory symptoms and infections or sick building syndrome, SBS, also have been observed in other environments such as day-care centers and offices (Harrison *et al.* 1992, Koskinen *et al.* 1995, Johanning *et al.* 1996, Li *et al.* 1997). In addition, some case studies have shown increase of asthmatic and other respiratory symptoms and even an outbreak of occupational diseases due to exposure to mold (Hodgson *et al.* 1998, Seuri *et al.* 2000).

The association between elevated levels of fungi and symptoms is supported by the observation that the symptoms decreased after the exposure had been eliminated (Koskinen *et al.* 1995, Jarvis and Morey 2001). However, there are several studies in which no correlation between fungal levels and symptoms or diseases has been observed (Strachan *et al.* 1990, Etzel *et al.* 1998, Garrett *et al.* 1998, Klánová 2000, Su *et al.* 2001). The distributions of fungal levels also overlap in the residences of symptomatic and non-symptomatic persons. No dose-response relationship between fungal levels and health effects has been observed or can be proposed as concluded also in the review of Verhoeff and Burge (1997) and hence no guidelines on fungal levels can be set based on health risk. The contradicting results concerning the associations between fungal concentrations and either occurrence of damage or symptoms of occupants is not so surprising in consideration of the diversity of sources of fungi and the huge variation in airborne fungal levels. This sets major demands on the development of methods which are both accurate enough to identify and to measure the exposure as well as distinguishing differences between normal and abnormal microbial exposure.

Table 2. The summary of studies determining associations between fungal levels and health effects.

STUDY	NUMBER AND TYPE OF SITES	STUDY DESIGN	METHOD	LEVELS OF AIRBORNE FUNGI	MAIN OBSERVATION
Waegemaekers *et al.*1989	36 residences	Damp (24) Reference (8)	6-stage impactor (MEA)	Damp: GM 192 cfu/m^3 Reference: GM 102 cfu/m^3	Fungal levels associated with respiratory symptoms.
Strachan *et al.* 1990	88 residences	Children with wheeze (34) Controls (54)	6-stage impactor (MEA)	Wheeze: <38 600 cfu/m^3 (MD 200-283) No wheeze: <41 300 cfu/m^3 (MD 224-271)	Median concentrations of viable fungi did not associate with wheeze.
Björnsson *et al.*1995	88 residences	Asthmatic adults (47) Controls (41)	Filter (total spores+viable)	Astmatics: GM 35*10^3 spores/m^3 (1.2-33 *10^4) 300 cfu/m^3 (100-3300) Controls: GM 25*10^3 spores/m^3 (1.1-57 *10^4) 300 cfu/m^3 (100-1300)	Spore levels higher in homes of asthmatics.
Dotterud *et al.*1995	38 residences	HDM-sensitized and control children (19/19)	BIAP Slit sampler (V-8 agar)	HDM-sensitized: <210 cfu/m^3 Controls: <80 cfu/m^3	Fungal levels higher in homes of HDM-sensitized children. High fungal levels related to high indoor RH.
Li *et al.*1995	92 residences	Asthmatic children (42) Atopic children (20) Controls (26)	6-stage impactor (MEA)	Asthmatics: GM 565-659 cfu/m^3 Atopics: GM 411-464 cfu/m^3 Controls: GM 602-608 cfu/m^3 Outdoor: GM 449-668 cfu/m^3	Concentrations in homes of asthmatics and controls higher than those in the homes of atopics.
Dill and Niggemann 1996	20 residences	Children with allergic diseases	RCS (MEA+Czapek Dox)	<13->4000 cfu/m^3	No correlation between fungal exposure and specific IgE to fungi.
Senkpiel *et al.*1996	7 residences	Asthmatics allergic to molds (7)	FH 2 impactor (Sabouraud-glucose agar)	100-1000 cfu/m^3	Levels much higher indoors than outdoors.
Etzel *et al.*1998	40 residences	Infants with pulmonary hemorrhage (10) Controls (30)	Filter: total and viable (Czapek Dox, MEA, DG18)	Patients: AM 29 227 cfu/m^3 Controls: AM 707 cfu/m^3	No association with total mean levels, but with selective fungi.
Garrett *et al.*1998	80 residences	Asthmatics (43) Non-asthmatics (37)	1-stage impactor (MEA)	<20-54749 cfu/m^3 (MD 812)	No association between mean levels and asthma.

Continued

Table 2. continued

STUDY	NUMBER AND TYPE OF SITES	STUDY DESIGN	METHOD	LEVELS OF AIRBORNE FUNGI	MAIN OBSERVATION
Johanning *et al.* 1999	2 residences	Mold damaged Control	1-stage impactor (MEA) Filter	Damaged: 1993->7069 cfu/m^3 1.8-6.6 *10^5 spores/m^3 Control: 194-336 cfu/m^3 3.7-4.7 *10^3 spores/m^3	Higher concentrations associated with respiratory symptoms.
Klánová 2000	Residences and offices 68 rooms:	A) no complaints + no mold (20) B) complaints + no mold (20) C) no complaints + visible mold (10) D) complaints+ visible mold (18)	RCS Plus aeroscop (YMA)	A) 0 - 230 cfu/m^3 (AM 78) B) 0 - 140 cfu/m^3 (AM 58) C) 60 - 3190 cfu/m^3 (AM 1033) D) 120 - 17930 cfu/m^3 (AM 2476)	Health complaints did not correlate with total concentrations of viable fungi.
Ross *et al.* 2000	44 residences	Asthmatics (57)	6-stage impactor	0-48760 cfu/m^3 (MD 1560)	Higher levels indicate association with emergency room visits.
Dharmage *et al.* 2001	485 residences	Random sample (349) Homes of asthmatics (136)	2-stage impactor (PDA)	37-7619 cfu/m^3 (MD 549)	High levels associated with increased BHR, but lower risk of being sensitized to fungi.
Jarvis and Morey 2001	2 apartment buildings	Moldy building Control building Effect of renovation	Volumetric culture plate impactor (MEA, DG18)	Before renovation: AM 330-340 cfu/m^3 After renovation: AM 41-96 cfu/m^3 Control: AM 50-316 cfu/m^3	Renovation of the building decreased both levels of fungi and prevalence of symptoms
Su *et al.* 2001	35 residences	Asthmatics (23) Controls (12)	Burkard (MEA)	AM 6798-20552 cfu/m^3	Airborne levels and flora not associated with asthma.
Harrison *et al.* 1992	15 offices	SBS and airborne microbes	6-stage impactor (MEA)	2-978 cfu/m^3 (MD 26-277)	SBS and microbial levels associated within buildings with similar ventilation systems.

Continued

Table 2. continued

STUDY	NUMBER AND TYPE OF SITES	STUDY DESIGN	METHOD	LEVELS OF AIRBORNE FUNGI	MAIN OBSERVATION
Koskinen *et al.* 1995	2 day-care centers	Moisture damaged, Reference	6-stage impactor (MEA)	Damaged: 120-430 cfu/m^3 Reference: 29-64 cfu/m^3	Exposed children had more symptoms and infections. After the exposure ended occurrence of symptoms decreased and infections disappeared.
Johanning *et al.* 1996	2 office buildings	Employees in water damaged office (53) Controls (21)	6-stage impactor (MEA, MEA + sucrose, Czapek)	Normal sampling: 48-116 cfu/m^3 Aggressive sampling: 302->20*10^6 cfu/m^3	Fungal levels from aggressive sampling associated with symptoms.
Li *et al.*1997	28 day-care centers	Air quality and health employees (264)	1-stage impactor (MEA)	Indoor: GM 1212 cfu/m^3	Total concentrations associated with SBS.
Hodgson *et al*. 1998	2 office buildings	Moisture damage in relation to health Case report	SAS PBI 1-stage impactor (MEA)	Indoor: 24-8000 cfu/m^3 Outdoor: 161-1650	Case description
Seuri *et al.* 2000	A hospital	Moisture damage in relation to health Case report	6-stage impactor	<1400 cfu/m^3	Case description

2.6.4 Differences in fungal flora between moisture damaged and reference buildings

Although differences in mean fungal levels between moisture damaged and reference buildings have not always been found, differences in microbial composition of air samples have commonly been noted. These observations are listed in Table 3, which shows the studies where certain fungal genera or microbial groups have been associated with either moisture damage of building or a certain health effect. For example, higher concentrations of *Aspergillus, Cladosporium*, *Penicillium*, non-sporulating fungi (including basidiomycetes) or yeasts have been observed in buildings with moisture damage or with visible mold growth than in reference buildings (Strachan *et al.* 1990, Pasanen 1992, Pasanen *et al.* 1992c, DeKoster and Thorne 1995, Garrett *et al.* 1998) (Table 3). In the study of Miller *et al.* (2000), the total concentrations of viable fungi were similar in residences with severe and mild mold damage, but the presence of severe damage could be seen in the higher prevalence of fungal species not present in the outdoor air (Miller *et al.* 2000).

Occurrence of certain fungi in air has also been associated with dampness or mold growth in buildings (Table 3). *Aspergillus versicolor* has been observed frequently in the air of damaged buildings (Hodgson *et al.* 1998, Jarvis and Morey 2001). *Stachybotrys* has been noted to occur in a moisture damaged building, but not in the control building (Johanning *et al.* 1999). In addition, several other genera different from outdoor air have been found, but their occurrence has not been reported to indicate moisture damage. In general, the dominant genera in air have usually been reported, but the value of rare findings as indicators of moisture damage has not been emphasized. However, a list of damage-associated fungi and bacteria has been published as a result of an expert meeting (Samson *et al.* 1994). This is based on empirical observations, but little published data are available about the frequencies or other characteristics of these microbes in building environments. The list of “indicator microbes”, or microbes that do not belong to the normal flora but the presence of which may indicate mold growth is as follows: *Trichoderma, Exophiala, Phialophora, Ulocladium, Stachybotrys, Fusarium, Wallemia, Aspergillus versicolor, Aspergillus fumigatus,* actinobacteria, gram-negative bacteria and yeasts (e.g. *Rhodotorula* and *Sporobolomyces*) (Samson *et al.* 1994). Furthermore, the occurrence of different microbes especially in the air should be

weighted differently, as some microbes, such as *Stachybotrys chartarum, Fusarium* and *Chaetomium* are seldom found airborne due to their spore size and spore formation (see section 2.4.). However, even these fungi can occasionally be present in abundance in air samples, especially when the fungi are growing prominently in a damage site (Hunter *et al.* 1988, Etzel *et al.* 1998, Johanning *et al.* 1999).

Table 3. Summary of the associations between microbial flora and moisture or mold damage or health effects.

GENUS or GROUP	**FOUND TO INDICATE / RELATE TO MOISTURE or MOLD DAMAGE**	**FOUND TO BE RELATED TO SYMPTOMS**
Actinobacteria	Nevalainen *et al.* 1991	
Aspergillus	Pasanen 1992 DeKoster and Thorne 1995	Li *et al.* 1997
Aspergillus versicolor	Jarvis and Morey 2001	Hodgson *et al.* 1998
Cladosporium	Garrett *et al.* 1998 Pasanen *et al.* 1992c Pasanen 1992	Garrett *et al.* 1998 Dharmage *et al.* 2001
Penicillium	DeKoster and Thorne 1995	Cooley *et al.* 1998, Garrett *et al.* 1998 McGrath *et al.* 1999 Dharmage *et al.* 2001
Non-sporulating fungi (includes e.g. basidiomycetes)	Strachan *et al.* 1990	Strachan *et al.* 1990
Stachybotrys	Johanning *et al.* 1999	Johanning *et al.* 1996 Etzel *et al.* 1998 Johanning *et al.1999*
Sporobolomyces		Seuri *et al.* 2000
Yeasts	Pasanen *et al.* 1992c Pasanen 1992	
Cladosporium, Epicoccum, Aureobasidium, yeast		Su *et al.* 1992

The reported associations between certain fungal genera or species and health effects are also listed in Table 3. These associations might provide hints about a causal relationship to health effects, but these connections have not been established at present. Elevated levels of *Penicillium* have been associated with SBS and asthmatic symptoms (Cooley *et al* 1998, Garrett *et al.* 1998, McGrath *et al.* 1999, Dharmage *et al.* 2001), while higher levels of *Cladosporium* have been associated with asthmatic symptoms and allergies (Garrett *et al.* 1998, Dharmage *et al.* 2001). Exposure to airborne S*tachybotrys* has been suggested to cause acute pulmonary hemorrhage in infants (Etzel *et al.*1998) and respiratory symptoms and disorders of central nervous

system (Johanning *et al.* 1996, Johanning *et al.* 1999). In the case study of Hodgson *et al.* (1998), *Aspergillus versicolor was* noted to dominate the mycoflora in offices where occupants were suffering from asthmatic and other respiratory symptoms, whereas exposure to *Sporobolomyces* in a hospital has been associated with a cluster of occupational diseases, including asthma, rhinitis and alveolitis (Seuri *et al.* 2000).

2.6.5 Airborne concentrations of bacteria

Concentrations of viable bacteria in residences vary between 10-10^4 cfu/m^3 (Nevalainen 1989, Nevalainen *et al.* 1991, Macher *et al.* 1991, Reponen *et al.* 1992, DeKoster and Thorne 1995, Rautiala *et al.* 1996, Ross *et al.* 2000, Pessi *et al.* 2002). Bacterial indoor air levels in residences are typically higher than outdoor air levels (Nevalainen 1989). Concentrations of viable bacteria have not been associated with moisture damage, but have been associated with the number of people living or occupying the residences (Nevalainen 1989, Nevalainen *et al.* 1991, DeKoster and Thorne 1995). The main genera found in the indoor air belong to *Micrococcus* and *Staphylococcus* groups (Nevalainen 1989, Górny *et al.* 1999), while the outdoor air may be dominated by gram-negative bacteria such as *Pseudomonas* (Nevalainen 1989). Associations have been found between asthma and levels of total, gram-positive and gram-negative bacteria in indoor air (Björnsson *et al.* 1995, Ross *et al.* 2000).

The occurrence of actinobacteria has been connected with the presence of mold damage (Nevalainen *et al.* 1991) and they are regarded as moisture indicative bacteria (see section 2.6.4). Actinobacteria are not of human origin, but they are abundant in the soil and grow also in moistened building materials (Andersson *et al.* 1997b). The airborne concentrations of viable actinobacteria in mold damaged buildings have varied between <4-154 cfu/m^3 (Nevalainen *et al.* 1991, Rautiala *et al.* 1996), while actinobacteria were not found in the reference houses (Nevalainen *et al.* 1991). The level of actinobacteria in indoor air has been noticed to be affected by the actinobacteria growth in the insulated external wall (Pessi *et al.* 2002). However, in farmhouses, actinobacteria occur commonly where they originate from barns and stables (Kotimaa *et al.* 1984, Nevalainen 1989, Pasanen *et al.* 1989). During the dismantling of moldy structures, actinobacterial levels air can be increased up to 10^4 cfu/m^3 in the indoor air (Rautiala *et al.* 1996).

2.6.6 Size distributions of airborne microbes

About 70-90% of the viable fungi in indoor air have been estimated to be in the respirable size fraction (<4.7 µm) (Li and Kuo 1994, DeKoster and Thorne 1995), whereas the corresponding number for viable bacteria is 50-60% (DeKoster and Thorne 1995). The median aerodynamic diameter is typically 3.4 µm for bacteria (Macher *et al.* 1991) and 2.0-3.0 µm for fungi in indoor air (Macher *et al.* 1991, Reponen 1995). The aerodynamic diameters of actinobacterial spores range from 0.6 to 1.3 µm (Reponen *et al.* 1998).

The highest concentrations of airborne viable fungi are usually in the size range of 2-3 µm (Rautiala *et al.* 1996), and the highest increase in concentration during the demolition of moldy structures was also seen in this size fraction (Rautiala *et al.* 1996). According to Górny *et al.* (1999), fungi and bacteria occur mostly as single particles in indoor air, but bacteria may form aggregates with tobacco smoke particles. The size of fungal spores increases with increasing air humidity (Reponen *et al.* 1996). This small increase in spore size, however, does not significantly affect the respiratory deposition of fungal spores (Reponen *et al.* 1996).

2.7 Microbes on surfaces, building materials and house dust

2.7.1 Fungal concentrations and flora in house dust

House dust samples have been suggested to provide a readily available way to obtain an integrated sample over a long period of time to reflect long-term exposure conditions (Flannigan 1997, Dillon *et al.* 1999). The concentrations and flora of viable fungi in dust are usually analyzed with a dilution method, from either dust vacuumed from carpets (mainly 1 m^2, 2 min) and mattresses (Verhoeff *et al.* 1994a, Dales *et al.*1997, Ellringer *et al.* 2000, Koch *et al.* 2000) or from the dust bag of the vacuum cleaner of the resident (Miller *et al.*1988). Also, direct spread of the dust on the nutrient medium has been used (Gravesen *et al.* 1986, Wickman *et al.* 1992). The methods to collect house dust and isolate culturable microbes have been reviewed by Macher (2001).

Concentrations of viable fungi in dust vary from 10 to 10^8 cfu/g (Miller *et al.* 1988, Verhoeff *et al.* 1994a, Dales *et al.* 1997, Koch *et al.* 2000, Ellringer *et al.* 2000). The most common genera or groups detected are typically *Penicillium*, yeasts, *Aspergillus*, *Cladosporium* and *Alternaria* (Miller *et al.* 1988, Wickman *et al.* 1992, Verhoeff *et al.* 1994b, Koch *et al.* 2001). These are the same ubiquitous fungal genera found also in the air. However, the fungal flora in the house dust may also differ from that present in air, e.g. *Mucor, Wallemia* and *Fusarium* have been found frequently in dust samples, but rarely in air (Gravesen *et al.* 1986, Miller *et al.* 1988, Ren *et al.* 1999). According to Koch *et al.* (2000), the fungal levels and flora in house dust are influenced by the outdoor air fungi and thus show a corresponding seasonal variation, but this was not seen in the study of Ren *et al.* (1999).

High levels of viable fungi, 120 000 cfu/g and 350 000 cfu/g, in dust have been suggested to indicate intramural sources of fungi by the working group of Commission of European Communities (DG18, dilution) (1993) and Koch *et al.* (2000), respectively. Other proposed limits to indicate indoor sources are based on the 95th percentiles of fungal concentrations by Koch *et al.* (2000); for *Alternaria* (10 000 cfu/g), *Aspergillus* (50 000 cfu/g), *Cladosporium* (30 000 cfu/g), and *Penicillium* (95 000 cfu/g) determined between November and May.

Mold and dampness have been associated with elevated levels of viable fungi in dust (Wickman *et al.* 1992, Dales *et al.* 1997), whereas in the study by Verhoeff *et al.* (1994b) this association was not significant. Instead, the type of flooring had a consistent effect on the concentrations of viable fungi in house dust (Verhoeff *et al.* 1994b). Even though a dust sample may represent an integrated sample over a long period of time, a single measurement of viable fungi in house dust does not provide reliable information of exposure due to the low reproducibility and differences in fungal genera compared to the air (Miller *et al.* 1988, Verhoeff *et al.* 1994a, Ren *et al.* 1999)

2.7.2 Microbes in surface samples

Airborne spores and cells may be deposited onto different surfaces in the indoor environment by gravitational settling or carried by wind currents. Thus, the spores found on indoor surfaces that are not regularly cleaned, may reflect the airborne mycoflora in

that indoor environment. Swab sampling from the surface will provide a rough estimate of the airborne flora. Concentrations of viable fungi on surfaces with no visible fungal growth or surfaces with no or minor moisture damage vary from being the under detection limit to approximately 2500 cfu/cm^2 (Ellringer *et al.* 2000, Lappalainen *et al.* 2001). The dominating genera have been *Penicillium* (Macher *et al.* 1991, Ellringer *et al.* 2000, Lappalainen *et al.* 2001) together with *Cladosporium*, yeasts (Macher *et al.* 1991, Lappalainen *et al.* 2001), *Aureobasidium* and *Alternaria* (Lappalainen *et al.* 2001). Interestingly, *Aspergillus* species have not been commonly found on undamaged surfaces. The fungal concentrations on surfaces nearby visible moisture damage have been reported as 3-260 cfu/cm^2, within the same range as nearby surfaces without damage (Lappalainen *et al.* 2001).

If there is water available on the surface, fungi will germinate and start to grow. In such situations, the numbers of viable fungi in swab sampling are several orders of magnitude higher than normal background, up to 10^6 cfu/cm^2 (Johanning *et al.* 1996, Jarvis and Morey 2001). The fungal genera observed on visibly damaged surfaces have been *Aspergillus versicolor* (Beguin and Nolard 1994, Lappalainen *et al.* 2001, Jarvis and Morey 2001), *Cladosporium, Penicillium, Ulocladium, Acremonium, Stachybotrys chartarum, Aureobasidium, Trichoderma* and *Scopulariopsis* (Beguin and Nolard 1994, Lappalainen *et al.* 2001). There are also studies in which *Stachybotrys* has been the dominating genus found on surfaces (Johanning *et al.* 1996).

Even damp surfaces supporting large populations of bacteria and yeasts or fungal growth do not necessarily result in higher microbial levels in the indoor air (Macher *et al*. 1991, Buttner and Stetzenbach 1993). *Stachybotrys* occurring on surfaces is often difficult to detect in the air, whereas easily sporulating genera such as *Aspergillus, Penicillium* and *Cladosporium* have commonly been observed in both types of samples (Cooley *et al.* 1998, Tiffany *et al.* 2000, Lappalainen *et al.* 2001). Hence, surface samples are often needed to confirm the findings, even though signs of contamination are seen in air mycoflora of air samples (Reynolds *et al.* 1990).

Bacterial concentrations on surfaces without visible growth have been noted to be under 4200 cfu/cm^2 (Lappalainen *et al.* 2001). Gram-positive rods have been found on both dry and damp surfaces (Macher *et al.* 1991), while gram-negative rods and

actinobacteria have been found in mainly damp or damaged areas (Macher *et al.* 1991, Lappalainen *et al.* 2001).

In addition to cultivation methods, fungal growth on surfaces can be determined from a tape sample by observing spores and hyphae with direct microscopy or with measurements of the rate of hydrolysis of fluoresceindiacetate to fluorescein by microbial enzymes (Bjurman 1999) or with detection of N-acetylhexosaminidase activity (MycoMeter) (Reeslev and Miller 2000).

2.7.3 Microbes in building materials

The concentrations of viable fungi in damaged materials vary typically between <45 – 10^8 cfu/g (Morey 1993, Andersson *et al.* 1997b, Etzel *et al.* 1998, Carlson and Quraishi 1999, Kujanpää *et al.* 1999, Johanning *et al.* 1999, Ellringer *et al.* 2000, Hodgson *et al.* 1998, Lappalainen *et al.* 2001, Pessi *et al.* 2002). Bacterial concentrations have shown an even wider range, <45-5*10^8 cfu/g (Andersson *et al.* 1997b, Kujanpää *et al.* 1999, Lappalainen *et al.* 2001, Pessi *et al.* 2002). Actinobacterial concentrations have been reported to cover 0-11% of the total bacterial concentration depending on the type of material (Kujanpää *et al.*1999, Reiman *et al.* 2000), in some cases being the dominant type of bacteria (Lappalainen *et al.* 2001). In the study of Andersson *et al.* (1997b), 13 bacterial genera were isolated from damaged building materials, with domination by gram-negative bacteria and *Mycobacterium* species. While moisture conditions may fluctuate in the microenvironments of a building, microbial growth is also a complex process regulated by the environmental factors. Therefore, moisture levels and microbial concentrations do not necessarily correlate well in building material samples (Meklin *et al.* 1999, Pasanen *et al.* 2000a).

The most common fungal genera found in material samples taken from damaged areas have been *Penicillium, Aspergillus* (Morey 1993, Andersson *et al.* 1997b, Etzel *et al.* 1998, Gravesen *et al.* 1999, Kujanpää *et al.* 1999, Ellringer *et al.* 2000, Reiman *et al.* 2000, Lappalainen *et al.* 2001) *Acremonium, Aspergillus versicolor*, *Cladosporium* (Ellringer *et al.* 2000, Reiman *et al.* 2000, Lappalainen *et al.* 2001), *Stachybotrys* (Andersson *et al.* 1997b, Gravesen *et al.* 1999, Johanning *et al.* 1999, Hodgson *et al.* 1998), *Chaetomium, Ulocladium* (Gravesen *et al.* 1999), yeasts and *Scopulariopsis*

(Reiman *et al.* 2000). In addition to these, a number of other genera or species are usually found in damaged materials. The dominant fungal genera found in the material samples are mainly similar to those seen in the air. There are, however, some genera such as *Stachybotrys,* that are not usually found in air (Miller *et al.* 2000, Tiffany *et al.* 2000). This is supported by the study of Rautiala *et al.* (1996), in which some infrequently found genera, such as *Absidia, Botrytis, Exophiala, Fusarium, Graphium, Mucor* and *Staphylotrichum*, were found in the damaged materials and also in the air during the dismantling of these materials. Building material samples, showing the actual growth at a given site, have been found to be useful in verifying the sources for the contamination, possibly seen in the air samples (Reynolds *et al.* 1990).

2.8 Other exposing agents

2.8.1 Structural components of microbes

Ergosterol

Ergosterol is the principal sterol present in membranes of hyphae and spores of filamentous fungi and thus provides a chemical marker for fungal biomass (Flannigan 1997). Ergosterol content in spores varies somewhat between different fungal species (Pasanen *et al.* 1999, Miller and Young 1997) and it is not a good indicator for yeasts which being non-filamentous, contain less ergosterol (Pasanen *et al.* 1999). Ergosterol is analyzed with the combination of gas chromatography-mass spectrometry (GC-MS) (e.g. Larsson and Saraf 1997) or with high-pressure liquid chromatography (HPLC) and GC-MS (e.g. Dales *et al.* 1997) or GC-MS-MS (e.g. Nielsen and Madsen 2000).

In house dust, ergosterol concentrations have been reported at <62 µg/g dust (Axelsson *et al.* 1995, Miller *et al.* 1988, Dharmage *et al.* 1999b) and the correlation with the counts of viable fungi has been good (Miller *et al.* 1988, Saraf *et al.* 1997). Miller and Young (1997) observed ergosterol in the air in range of 0.01-194 ng/m^3, while mean ergosterol values of 200 -3000 ng/m^3 were reported by Dales *et al.* (1997). Ergosterol was detectable only in about 5-25% of the air samples in these studies. The ergosterol concentrations have been higher in damaged materials (0.01-0.15 ng/g) than

in dry materials samples (nd) (Szponar and Larsson 2000) and correlated with concentrations of viable fungi (Pasanen *et al.* 1999).

Ergosterol concentrations have been found to vary seasonally in dust, being highest in winter (Dharmage *et al.* 1999b), while the variation is the opposite for culturable fungi. Ergosterol levels in dust have been associated with visible or reported mold and fitted old carpets, with pets and infrequent airing (Dharmage *et al.* 1999a, Dharmage *et al.* 1999b). However, in studies of Dales *et al.* (1997) and Lawton *et al.* (1998), visible mold areas or reported mold and water damage were not associated with ergosterol level in dust or in the air. While ergosterol has been suggested to estimate fungal exposure (Flannigan 1997), its relationship to the observed health effects are still obscure (Szponar and Larsson 2001).

Endotoxins

Endotoxins are biologically active lipopolysaccharide (LPS) molecules that form the outer membrane of gram-negative bacteria. The endotoxin activity can be measured with the *Limulus* amebocyte lysate assay (LAL) (e.g. Hines *et al.* 2000). Chemical determination is possible by analyzing the 3-hydroxy fatty acids of the lipid A, which is a component of LPS (Liu *et al.* 2000, Larsson and Saraf 1997). There is a correlation between determinations of endotoxin with LAL and 3-hydroxy fatty acid concentrations in dust (Saraf *et al.* 1997).

The median concentrations of airborne endotoxin have been 0.6-4.6 endotoxin units /m^3 (EU/m^3) in homes both with and without mold (Dales and Miller 1999, Park *et al.* 2000). In house dust, mean levels have varied between 18-50 ng/g (Su *et al.* 2001) and 44-105 EU/g (Park *et al.* 2000). Both air and house dust levels of endotoxin have seasonal and within-home variation (Park *et al.* 2000, Su *et al.* 2001). The airborne endotoxin levels in winter are dominated by indoor sources while the outdoor air contributes to the levels in other seasons (Park *et al.* 2000).

The endotoxin levels in air have been associated with air humidity and weakly with moisture sources (Park *et al.* 2000, Park *et al.* 2001), while endotoxin levels in dust have been not associated with home dampness (Gereda *et al.* 2001). Both air and dust

endotoxin levels have been associated with the presence of pets (Park *et al.* 2000, Gereda *et al.* 2001). The biocontamination of moisture damaged building materials could be shown as the difference in endotoxin and 3-hydroxy fatty acid levels compared to non-damaged material (Andersson *et al.* 1997b, Szponar and Larsson 2000). The role of endotoxin health related exposure in moisture damaged buildings remains unclear.

β(1→3)-Glucans

β(1→3)-Glucans are glucose polymers, which may originate from several sources, such as most fungi, some bacteria and plants. β(1→3)-glucans can be determined with glucan-reactive preparation of *Limulus* amebocyte lysate (LAL) (Rylander *et al.* 1992) and enzyme inhibition immunoassay (EIA) (Douwes *et al.* 1996).

The airborne levels of β(1→3)-glucan in residents have been reported to vary between 0-19 ng/m^3 (Thorne and Rylander 1998). The β(1→3)-glucan levels in moisture-damaged building materials have been 2.5-210 µg/g and 0.4 µg/g in non–damaged materials (Andersson *et al.* 1997b). The mean concentrations of β(1→3)-glucan in dust have been observed to vary from 1.55 to 2.22 µg/g and to positively associate with total culturable fungi in dust (Chew *et al.* 2001).

β(1→3)-glucan has been suggested to associate with respiratory symptoms in complaint buildings (Rylander *et al.* 1992, Rylander *et al.* 1998) and a decrease in β(1→3)-glucan levels (11.4 ng/m^3 → 1.4 ng/m^3) was observed due to renovation (Rylander 1997). However, the importance of β(1→3)-glucan as an exposing agent in moldy buildings remains to be clarified.

2.8.2 Microbial metabolites

Microbial volatile organic compounds

Microbes can produce volatile organic compounds, microbial VOC (MVOC) as end products of their metabolism. Volatile organic compounds are mainly sampled by drawing air with a pump through a small tube filled with one or several sorbents, on

which the compounds to be collected are adsorbed. In addition, VOCs can be sampled passively with sorbents on which the compounds diffuse or with whole air sampling. The compounds are desorbed from sorbents thermally or with liquid extraction. The analysis is generally performed with combination gas chromatography-mass spectrometry (GC-MS) (Batterman 1995).

MVOCs are responsible for the “smell of mold, musty or earthy odors”, often associated with microbial growth. Several compounds such as 1-octen-3 ol, geosmin, 3-methylfuran, 3-methyl-2-butanol, 2-pentanol, 2-hexanone, 3-octanone, 2-octen-1-ol, 2-methyl-isoborneol, 2-isopropyl-3-methoxypyrazine, 3-methyl-1-butanol, 2-heptanone and 3-octanol, have been suggested as indicators of microbial contamination in buildings (Ammann 1999, Korpi 2001). The production of MVOCs is dependent on the microbial species and growth conditions, but on the other hand, some metabolites are produced by several species and on more than one medium (Sunesson 1995, Fiedler *et al.* 2001).

MVOCs have been suggested as representing a method for detecting “hidden mold” (Wessen *et al.* 1999). None of the commonly reported MVOCs, however, are considered to be specific for microbial metabolism, but they are also released from moist building materials and constructions (Korpi 2001). In fact, no consensus on the relevant MVOCs exists (Pasanen 2001). Hence, the interpretation of MVOC results as a mold indicator is contradictory. Furthermore, the production of MVOC indicates only active growth. MVOCs are found in extremely low levels in indoor air and their role as possible causative agents of irritation symptoms is not yet clear (Pasanen *et al.* 1998, Korpi *et al.* 1999).

Microbial toxins

Mycotoxins are non-volatile, secondary metabolites of fungi, which may be produced due to competition with other microbes. Their chemical structures are quite diverse including polyketides, terpenes and indoles (Burge and Ammann 1999). Betina (1989) has estimated that over 300 mycotoxins are formed by 350 species. The most well known mycotoxins are aflatoxins, produced by *Aspergillus flavus*, thichothecenes, produced by *Fusarium* sp. and *Stachybotrys chartarum,* and ochratoxins, produced by

some species of *Penicillium* and *Aspergillus* (Betina 1989). Common to most mycotoxins is their acute toxicity, some of them being among the most toxic compounds known (Betina 1989, Hintikka and Nikulin 1998). Also, many bacteria may produce toxins (Stanier *et al.* 1977); actinobacteria are known to produce very potent secondary metabolites (Hodgson 2000).

Humans are exposed to mycotoxins mainly by ingestion of mycotoxin-contaminated food or by airborne exposure via mycotoxin-containing spores or other particles in moldy buildings or agricultural environments (Sorenson *et al.* 1987). Exposure to mycotoxins, especially *Stachybotrys* toxins, in buildings has been associated with adverse health effects (Johanning *et al.* 1996, Etzel *et al.* 1998, Jarvis *et al.* 1998, Johanning *et al.* 1999); even the occurrence of pulmonary hemorrhage in infants due to exposure to *Stachybotrys* mycotoxins in their homes has been proposed (Etzel *et al.* 1998, Jarvis *et al.* 1998). However, a recent literature review by Page and Trout (2001) concludes that there is not enough evidence to support a causal relationship between symptoms or illness among building occupants and exposure to mycotoxins.

The attempts to detect mycotoxins in indoor air have failed so far (Pasanen 2001), but both bacterial and fungal toxins have been detected in moisture damaged building materials (Andersson *et al.* 1997b, Gravesen *et al.* 1999, Nielsen *et al.* 1998, Nielsen *et al.* 1999, Tuomi *et al.* 2000). Mycotoxins are analyzed with thin layer chromatography (TLC), high pressure liquid chromatography (HPLC) or the combination of GC-MS (Burge and Ammann 1999).

2.8.3 VOC and formaldehyde

Emissions of formaldehyde and volatile organic compounds (VOCs) are enhanced by moist conditions within the building (Reponen *et al.* 1991, Tucker 1991) and thus these chemicals may be potential contaminants in moisture damaged buildings. The emissions may be increased due to chemical and microbiological deterioration caused by water (Batterman 1995). Volatile organic compounds are released from building materials and numerous other sources including paints, varnishes, detergents, personal use products, such as cosmetics and hair sprays, furniture and carpets and are also

present in polluted outdoor air, mostly due to motor vehicle emissions (Commission of European Union 1994, Batterman 1995).

The concept of total volatile organic compounds, TVOC, is used to characterize the total amount of volatile compounds in the air. However, TVOC can be measured with different techniques and can be defined in several ways (Mølhave *et al.* 1997).

The TVOC-values in new residences have been reported to vary between 0.21-9.60 mg/m^3, while their concentrations decreased after 5 months' occupancy to levels of 0.06-1.34 mg/m^3 (Tuomainen *et al.* 2001). In residences in general, levels of TVOC are usually <1mg/m^3 (Brown *et al.* 1994, Kostiainen 1995, Mølhave *et al.* 1997), but may even exceed 10 mg/m^3, though seldom over 25 mg/m^3 (Miller *et al.* 1988, Norbäck *et al.* 1995, Mølhave *et al.* 1997). The Nordic Scientific Consensus Meeting has concluded that the scientific literature is inconclusive with respect to TVOC being a risk for health and comfort effects in buildings (Andersson *et al.* 1997a), which is supported by Mølhave *et al.* (1997).

Elevated formaldehyde (HCHO) levels were earlier common in the indoor air due to the use particle board with a high emission rate of HCHO. It is still ubiquitous, but released less from the present particle boards. HCHO is emitted from pressed wood constructions, urea formaldehyde foam insulation, household cleaning agents, and smoking (IEH 1996). The levels of formaldehyde vary typically from 0.01 to 0.1 mg/m^3 in European homes and schools (IEH 1996). In new Finnish residences, formaldehyde levels have varied in the range of 1-27 µg/m^3, while they decrease after 5 months' occupancy to the level of 2-21 µg/m^3 (Tuomainen *et al.* 2001). In Swedish residences, levels have varied between <5-110 µg/m^3 and elevated levels were associated with nocturnal breathlessness (Norbäck *et al.* 1995).

2.8.4 House dust mites

The amount of house dust mites can be estimated with determination of the main allergens of house dust mites, *Der* f1 and *Der* p1, or the nitrogenous excretory product of mites, guanine, in the dust. The allergen measurements are usually performed with ELISA (van Strien *et al.* 1994, Julge *et al.* 1998, Custovic *et al.* 1999, Sporik *et al.*

1999) and the amount of guanine is determined with a semi-quantitative colorimetric test (Björnsson *et al.* 1995).

The mite allergen levels (*Der* p1 and *Der* f1) in house dust vary typically between <0.02-20 µg/g (van Strien *et al.* 1994, Julge *et al.* 1998, Custovic *et al.* 1999, Leung *et al.* 1998, Tunnicliffe *et al.* 1999, Ross *et al.* 2000, Su *et al.* 2001). However, high concentrations 60-200 µg/g can be found, especially in mattresses (Julge *et al.* 1998, Leung *et al.* 1998, Tunnicliffe *et al.* 1999). House dust mites are known to need high levels of indoor air humidity (RH>50%) and a warm temperature is favorable for them (O'Rourke *et al.* 1993, van Strien *et al.* 1994, Hirsch *et al.* 2000), but such associations are not always found (Su *et al.* 2001). The median concentrations of mite allergens did not associate with reported mold and symptoms or asthma (van Strien *et al.* 1994, Dales and Miller 1999, Ross *et al.* 2000). Even though house dust mite allergens are found in dust, it is possible that no allergens will be found in the air (Custovic *et al.* 1999)

House dust mite allergens are found virtually in all homes in moderate climate (van Strien *et al.* 1994, Leung *et al.* 1998), but less frequently in a cold climate. This low frequency of house dust mites in the Scandinavian climate was supported by a Swedish study, where house dust mites were found relatively infrequently, in only 13% of homes (Björnsson *et al.* 1995). Their presence was, however, associated with prevalence of asthma (Björnsson *et al.* 1995). In Finland, no *Der* p 1 was found in the floor dust of 30 residences (Raunio *et al.* 1998).

2.9 Mold-specific immunoglobulin G antibodies

Immunoglobulin G (IgG) antibodies are produced as a part of the human defense system. When foreign antigens such as microbes enter the human body the production of antibodies is induced. The presence of IgG antibodies demonstrates an exposure, not the development of a disease (Janeway and Travers 1994). Mold-specific IgG antibody levels have been shown to be a useful tool for exposure assessment in occupational environments, such as sawmills, agriculture, and handling and treatment of biowaste (Eduard *et al.* 1992, Erkinjuntti-Pekkanen 1996, Lappalainen *et al.* 1998, Bünger *et al.* 2000). In addition, elevated mold-specific IgG levels have been associated

with fungal exposure among the personnel of a water-damaged hospital (Seuri *et al.* 2000). These studies have shown IgG antibodies useful for adult populations with high exposure to microbes. In other indoor environments, such as schools, offices, and homes no or only a weak association between mold exposure and IgG-levels among adults and children have been detected (Johanning *et al.* 1996, Malkin *et al.* 1998, Makkonen *et al.* 2001, Taskinen *et al.* 2002).

2.10 Methods to characterize moisture damaged buildings

Moisture and mold growth are associated with several health effects (see section 2.1), but the causal agents and mechanisms are still poorly understood. Thus, in the search for causal agents it is important to characterize the differences between moisture damaged and non-damaged buildings together with epidemiological studies focusing on the associations between health effects and exposure. In the following section, the observed differences between damaged and non-damaged buildings are summarized.

In some studies, differences between moisture damaged and reference buildings could be seen in airborne concentrations of fungi (Gallup *et al.* 1987, Waegemakers *et al.* 1989, Verhoeff *et al.* 1992, Dharmage *et al.* 1999b, Johanning *et al.* 1999, Klánová 2000, Hunter *et al.* 1988), but there also are several studies suggesting the opposite or reporting inconclusive results (Strachan *et al.* 1990, Nevalainen *et al.* 1991, Pasanen *et al.* 1992c, Pasanen 1992, Dill and Niggemann 1996, Garrett *et al.* 1998). In several studies, airborne microbial flora has been different from outdoor air (DeKoster *et al.* 1995, Hodgson *et al.* 1998, Miller *et al.* 2000, Jarvis and Morey 2001) or concentrations of certain microbial genera or groups have been higher in moisture damaged buildings than in non-damaged buildings (Strachan *et al.* 1990, Nevalainen *et al.* 1991, Pasanen 1992, Pasanen *et al.* 1992c, Garrett *et al.* 1998). There are not enough data on concentrations and flora on non-damaged surfaces and building materials to reveal the difference between moisture damaged and reference buildings, because the information on surface and building material samples is mainly descriptive. These types of samples have been shown to give additional information on fungal flora in buildings (Cooley *et al.* 1998, Rautiala *et al.* 1998, Miller *et al.* 2000, Tiffany *et al.* 2000, Lappalainen *et al.* 2001). The association between moisture and mold damage and

elevated levels of fungi in dust is still contradictory (Wickman *et al.* 1992, Verhoeff *et al.* 1994b, Dales *et al.* 1997, Ren *et al.* 1999)

Some associations between levels of endotoxin, ergosterol, β(1→3)-glucans and other structural markers of microbial biomass, such as extracellular polysaccharides, EPS, and presence of mold or moisture problem have been indicated (Rylander 1997, Dharmage *et al.* 1999a, Douwes *et al.* 1999, Park *et al.* 2001), but little is known about their levels and the variations present in moisture damaged and reference buildings. MVOCs have been suggested to represent a mean for detecting hidden mold growth (Wessén *et al.* 1999), but the differences in levels and specific compounds between moisture and reference buildings remains to be clarified. Mycotoxins and bacterial toxins can be considered as possible causative agents of health effects, but because they can be measured only from the source itself, seldom directly from air, their use in exposure assessment is indirect and so far inconclusive. Nevertheless, in the source characterization, the occurrence of mycotoxins in building materials verifies the severity of the mold damage. As the health effects of mold exposure are evident, but the causative agents still remain unclear, further studies are needed to characterize the exposure and the differences in the indoor environment that are caused by moisture damage of buildings.

3 AIMS OF THE STUDY

This thesis combines the results of the original studies I-VI. The overall aim of the current study was to characterize the differences between moisture damaged buildings and reference buildings. The detailed aims of the study were to find answers to the following questions:

1. How does the indoor quality of a moisture damaged building differ from that of a reference building? (I,II,V,VI)

2. Can a building with mold problems be identified with cultivation of microbes from environmental samples from the indoor environment? (I,II,V,VI)

3. Is there significant temporal and spatial variation in concentration of airborne fungi in residential environments? What is the recommended sample size required for the characterization the fungal concentration of a residence? (III)

4. Are certain microbes associated with certain moisture damaged building materials?(IV)

5. Is there an association between fungal findings in buildings and microbial-specific IgG-levels of the occupants? (V, VI)

4 MATERIAL AND METHODS

4.1 The buildings studied

The following types of buildings were investigated: residences, day-care centers and schools. The buildings studied were classified as index and reference buildings according to technical criteria concerning moisture damage. Index buildings had frequent signs of moisture in structures, mostly due to leaks in roofs and plumbing, missing or inadequate drainage and construction defects in the insulation. Visible mold was usually also observed in some parts of the index buildings. The reference buildings had no water damage or only a few minor signs of moisture, which were assessed to be consequences of normal aging of the building.

Studies I and II were carried out in nine index buildings with moisture and mold problems: six residences and three day-care centers. Four of the residences were apartments and two were detached houses. For each index building, a reference building was chosen matching the age, site, use, construction materials and architecture. Hence, a total of 12 residences and six day-care centers were included in the study. Each index building and its reference were located in the same town and the occupants represented similar socio-ecomic status of the population.

Study III was conducted in two residences. The index residence was a single-family house with observed moisture problems; the reference residence was a single-family house with no such problems. The houses were matched for building type, age, site, use, occupancy and architecture. Both houses were used as day-care homes and three to five children under seven years of age were present during the working hours of each sampling day.

Study V was carried out in twelve index residences with moisture or mold problems and twelve reference residences without such problems. The residences consisted of single-family houses, row houses and apartment buildings. The data of study V have been published only partly; additional results of the study are included in the results section.

Study VI was conducted in two primary schools, a moisture-damaged index school and a non-damaged reference school.

In addition, several databases (Study I, III, V, Rautiala *et al.* 1996 and others) were merged to estimate the overall range of concentrations of viable fungi in index residences (III) and to show cumulative distributions of concentrations in index and reference residences.

4.2 Building material samples

In study IV, 1140 samples of building materials were either collected during study visits or sent for microbial analysis to KTL, National Public Health Institute, Finland, or the University of Kuopio, Finland. All samples were collected from materials with visible damage. Materials consisted of wood (n=451), paper (n=49), non-wooden building boards i.e. gypsum boards (n=46), ceramic products (n=163), mineral insulation materials (n=220), paints and glues (n=86), and plastics (n=125).

4.3 Technical investigations

Moisture and mold damage of the buildings were observed during a technical investigation which included a thorough walk-through to visually observe signs of moisture faults, such as signs of water leakage, detaching or discoloring of materials. Tenants or personnel were interviewed to ascertain the damage history (I-III, V-VI). In studies III and VI, buildings were inspected by a trained civil engineer using a standardized protocol (Nevalainen *et al.* 1998) and the investigations were supplemented with surface moisture recorders (Doser BD-2 and BS-2). The temperature and relative humidity of the indoor and outdoor air were recorded (HMI 31 and HM34, Vaisala, Helsinki, Finland).

4.4 Study populations

In study V, 25 persons living in moisture damaged houses and 17 persons living in reference houses were studied for their mold specific immunoglobulin G (IgG) antibodies from the sera.

In study VI, 181 children who had a doctor-diagnosed asthma or had experienced wheezing or cough symptoms during the previous 12 months provided a blood sample for IgG analyses. In addition, their health status was examined with a questionnaire. The children were a subpopulation of a total of 622 children participating in a questionnaire study done in a moisture damaged and a reference school in Siilinjärvi (Taskinen *et al.* 1999).

4.5 Sampling and analysis of indoor air pollutants

4.5.1 Airborne microorganisms

The samples for airborne viable microorganisms were collected with a six-stage impactor (Andersen 10-800; Graseby Andersen, Atlanta, Georgia, USA) (I-III,V-VI). This device was chosen for its good collection characteristics. A method based on detection of viable micro-organisms was chosen in order to characterize microbial flora. Impactor samples were taken in the middle of the room at a height of 1 m with the flow rate of 28.3 l/min (I-III,V-VI). In studies I and II, conducted in the fall and winter and in study III, conducted only in winter, one outdoor air sample for each sampling day were taken. In studies V and VI, samples were taken only in winter and no outdoor air samples were taken, because in a subarctic climate outdoor air levels of microbes in winter are low due to the snow cover and hence the contribution of the outdoor air to indoor air microbial concentrations is negligible (Reponen *et al.* 1992).

The number of rooms sampled, sampling periods and samples per day per room are presented in Table 4. Sampling time was 10 min for the mesophilic bacteria (II,VI) and fungi (I-III,V-VI) and 15 min for the thermotolerant bacteria (II). Concentrations

were calculated using Andersen correction table for multiple impactions on individual deposition sites (Andersen 1958).

Table 4. Number of rooms sampled, sampling periods and samples per day per room.

Number of	Study I-II	Study III	Study V	Study VI
Rooms sampled	2-4	2	2-6	17-20
Sampling periods	2	6	1-2	1
Samples / room	1	3	1	1

The growth media and incubation conditions used are presented in Table 5. All growth media were supplemented with either antibiotics or fungicides to suppress the growth of bacteria and fungi, respectively. After incubation, the number of fungal and bacterial colonies were counted. Actinobacteria -type bacterial colonies were counted separately (II,VI) and fungal colonies were identified morphologically by genus using an optical microscope (I-VI,IV).

4.5.2 Surface samples

In study I, the aim of the surface samples was to obtain qualitative information on the genera of fungi that are attached to the surfaces of the buildings. Surface samples (100 cm^2) were taken from interior surfaces with a sterile swab into sterile water and suspensions were plated on malt extract growth media (MEA) (Difco, Detroit, Michigan, USA). The fungal genera were determined. In studies V and VI, surface samples were taken from visibly damaged surfaces in order to determine possible fungal growth and genera. Samples (100 cm^2) were taken with a sterile swab into sterile Tween 80 dilution buffer (distilled water with 42.5 mg/L KH_2PO_4, 250 mg/L $MgSO_4$ x $7H_2O$, 8 mg/L NaOH and 0.02% Tween 80 detergent). Reference samples (200 cm^2) were taken from corresponding, undamaged surfaces (VI). A series of dilutions were prepared, plated on growth media and incubated as shown in Table 5. After incubation, the number of fungal and bacterial colonies was counted. Actinobacteria -type bacterial colonies were counted separately (VI) and fungal colonies were identified morphologically by genus using an optical microscope (I, V-IV).

Table 5. The growth media and incubation conditions.

Sample type	Fungi			Bacteria		
	Growth media	**Temp. (°C)**	**Time (days)**	**Growth media**	**Temp. (°C)**	**Time (days)**
Impactor	MEA[a] (I-II,V) 2%MEA[b] (III,V-VI) DG18[c] (III,VI)	20-25 (I-III,V-VI)	5-7(I-III,V-VI)	TYG[a, d] (II,VI) Half-strength nutrient[a] (II)	20-23 (II,VI) 55 (II)	5 (II, VI), 14* (VI) 2-3 (II)
Sedimentation	MEA[a] (I)	20-23 (I)	5-7 (I)			
Surface	MEA[a] (I,V) 2%MEA[b] (V-VI) DG18[c] (VI)	20-25 (I,V-VI)	5-7(I, V-VI)	TYG[a](VI)	20-23 (VI)	5 (VI), 14* (VI)
Material	MEA[a] (II,IV) 2%MEA[b] (IV,VI) DG18[c] (IV,VI)	20-25 (II,IV,VI)	5-7(II, IV,VI)	TYG[a] (VI) Caseinate-propionate (II) Half-strength nutrient[a] (II)	20-23 (VI) 20-23 (II) 55 (II)	5 (VI), 14* (VI) 5-7 (II) 2-3 (II)
House dust	MEA[a] (I)	20-23 (I)	5-7 (I)			

MEA = malt extract agar
DG18 = dichloran glycerol agar
TYG = tryptone yeast glucose agar
[a] Difco, Le Pont de Claix, France
[b] Biokar, Beuvais, France
[c] Oxoid, Basingstoke, Hampshire, England
*The number of 'dry' actinobacteria–type colonies was counted after 14 days.

4.5.3 Building material samples

Material samples were taken from obviously contaminated materials of indoor surfaces (e.g. wallboard) and structural materials (e.g. insulation). Samples were weighed, homogenized, and extracted with dilution buffer (distilled water with 42.5 mg/l KH_2PO_4*7 H_2O, 250 ml/l $MgSO_4$, 8 mg/l NaOH and 0.02% Tween 80 detergent). Suspensions were held in an ultrasonic bath (FinnSonic MO3/m) for 30 minutes and in a shaker (KS125 basic, IKA Labotecknik) for 60 minutes (400-600 r/min) (II, IV, VI). Dilution series were made, plated on growth media and incubated as shown in Table 5.

After incubation, the number of fungal and bacterial colonies were counted. Subsequently, fungal colonies were identified morphologically by genus using an optical microscope. Concentrations (cfu g^{-1}) were calculated using the fresh weight of the sample. (II,IV-IV)

Bacterial and fungal concentrations were obtained from dilutions which produced separate colonies on the agar plate (II, IV-VI). If additional fungal genera or actinobacteria colonies were detected on dilutions where growth was so dense that counting of individual colonies was impossible, the occurrence of these genera or actinobacteria was recorded. This information was included in data representing the presence or absence of each genus, species or group (IV). When counting of individual colonies was impossible for all dilutions, due to high fungal or bacterial concentrations, the total concentration was estimated by assuming that the number of colonies produced by the highest dilution was 150 colonies for fungi ($1.5x10^8$ cfu g^{-1}) and 650 colonies for bacteria ($6.5x10^7$ cfu g^{-1}) (IV, VI).

4.5.4 House dust samples

In study I, house dust samples were obtained by using the vacuum cleaner of each building studied. The vacuum cleaner was fitted with an unused dust bag before collection. The collection time was 2-4 weeks. Samples were taken during fall and winter. Dust samples of 1 g were suspended into 100 fold dilution buffer (distilled water with 42.5 mg/l KH_2PO_4, 250 mg/L $MgSO_4$ x $7H_2O$, 8 mg/l NaOH and 0.02% Tween 80 detergent), and the sample was shaken for 60 s. The suspension was filtered, diluted

and plated on malt extract agar (Table 5). The samples were incubated in the dark at 20-23°C for 5-7 days. The fungal concentrations and flora were determined.

4.5.5 House dust mites

Samples for house dust mites analyses were collected from beds, sofas and other padded furniture with a vacuum-cleaner onto filter paper. One combined sample was taken for each building. The presence of house dust mites was analyzed with Acarex-test (Werner & Mertz, Reinbek, Germany) (II).

4.5.6 Formaldehyde and TVOC

Formaldehyde concentrations were determined with the chromotrophic acid method (NIOSH 1974) (II). TVOC-samples were taken passively with Tenax-TA-resin tubes (Dietz and Cote 1982). TVOC sampling was started during the microbial sampling and maintained for one week. TVOC analyses were performed with the gas chromatography-mass spectrometer method modified by Pasanen *et al.* (1990) (II).

4.6 Serum samples for mold specific IgG

Mold specific IgG levels were studied as a possible biomarker of exposure. The serum samples were stored at -20°C (VI) or -70°C (V) until all the serum samples were collected. After the collection was completed, the antibody determinations of all the samples were analyzed as one set. Analyses were done blinded in random order. (V-VI)

In study V, 24 fungal strains were selected for antibody determination on the basis of the results from environmental sampling of the study, whereas in study VI, serum IgG antibodies were determined to 21 fungal strains, and three actinobacterial strains, which were considered to cover the major microflora of the living environments. The microbes used in the antibody analyses are shown in Table 6.

Table 6. The list of microbes and study number (in roman numerals) included in the investigation of microbe-specific IgG-antibodies.

Microbe specific IgG antibodies
Acremonium kiliense (VI)
Acremonium atrogriseum (VI)
Alternaria alternata (V)
Aspergillus niger (V)
Aspergillus fumigatus (V, VI)
Aspergillus umbrosus (V)
Aspergillus versicolor (V, VI)
Aureobasidium pullulans (V,VI)
Botrytis cinerea (V)
Cephalosporium curtipes (V)
Cladosporium cladosporioides (V, VI)
Chaetomium globosum (VI)
Eurotium amstelodami (VI)
Fusarium avenaceum (V)
Fusarium oxysporum (VI)
Geotrichum candidum (V, VI)
Humicola grisea (V)
Mucor circinelloides (VI)
Paecilomyces variotii (V, VI)
Penicillium brevicompactum (V)
Penicillium frequentans (V)
Penicillium glabrum (VI)
Penicillium notatum (VI)
Phialophora bubakii (V)
Phoma macrostoma (VI)
Rhizopus nigricans (V, VI)
Rhodotorula glutinis (V,VI)
Scopulariopsis brevicaulis (V)
Sporobolomyces salmonicolor (V, VI)
Stachybotrys chartarum (V, VI)
Streptomyces albus (VI)
Streptomyces griseus (VI)
Streptomyces halstedii (VI)
Trichoderma citrinoviride (V,VI)
Tritirachium roseum (VI)
Ulocladium atrum (V)
Wallemia sebi (V)

Intracellular antigens for antibody determination were prepared as follows (V,VI). Fungi were cultured in Roux bottles on mycological peptone broth (2% malt extract, 1% mycological peptone, 4% glucose in sterile water) for 7 days. Harvested microbial mass was washed three times with phosphate buffered saline (PBS), pH 7.4. After

autoclaving, disrupting by a homogenizer and an ultrasonic treatment, microbial homogenates were centrifuged. After the filtration through 0.4 µm pore size, filtered supernatants were stored at -70°C until used as antigens in the enzyme-linked immunosorbent assay (ELISA). Alkaline phosphatase conjugated anti-human IgG (Sigma, St. Louis, MO, USA) was used in ELISA as antiserum for the detection of IgG antibodies in the sera samples. (V,VI)

The working dilutions of antigens were determined from the titration curves for each microbe separately by using IgG positive sera diluted 1:100. Serum immunoglobulin G (IgG) antibody concentrations were determined by an enzyme-linked immunosorbent assay (ELISA) (Laitinen *et al.* 1999). In the ELISA, absorbance values of a test serum were compared to those of a pooled control serum collected from adults positive to a few molds/microbes. The control serum was used on each microtiter plate. Absorbances given by a test serum are expressed as percentages from the absorbances of the corresponding microbes given by the pooled control serum. (V,VI)

4.7 Health questionnaires

The questionnaire in study VI was based on the Örebro-questionnaire (MM-40) (Andersson 1998) supplemented with additional questions on respiratory infections (common cold, tonsillitis, otitis, sinusitis, bronchitis, pneumonia) (Susitaival and Husman 1996), use of health care services, and background factors, such as housing conditions, pets, passive smoking and parental profession. The questions on asthmatic symptoms (shortness of breath, wheezy chest, attacks of shortness of breath with wheezing, dry cough apart from coughing with a cold or chest infection) and doctor diagnosed asthma were similar to those used in study of Timonen (1997). The questionnaires were filled in by the parents.

4.8 Ethics

The study protocol of study VI was approved by the Ethics Committee of the University Hospital of Kuopio. Information about the study was delivered in meetings open to the personnel of the schools and the parents of the schoolchildren. Written consents for

drawing the blood samples from the schoolchildren were obtained from the parents of each child.

4.9 Statistical analysis

The distributions of fungal genera were not normally distributed, and hence non-parametric tests were mainly used. The statistical tests used are summarized in Table 7. SPSS (I-II, V) and SAS (III-IV, VI) statistical packages were used for analyses (SPSS Inc. 1988, SAS Institute Inc. 1990).

Table 7. Statistical methods used.

Use	**Method**
Difference in microbial concentrations	Wilcoxon signed rank test (I-III) Wilcoxon rank test (III,V-VI) Paired t-test (III) Two sample t-test (III) Kruskal Wallis one way analysis of variance (IV) Dunn's Post hoc test (IV)
Difference in microbial frequencies	McNemar test (I) Chi Square test (IV)
Correlation between media	Pearson's correlation test (III) Spearman rank correlation analysis (VI)
Correlation between fungal and bacterial concentrations	Spearman rank correlation analysis (IV)
Temporal and spatial variation of fungal concentrations	Anova (III)
Association between materials and genera	Logistic regression (binary data) (IV)
Concurrent occurrence of two genera	Chi Square test (binary data) (IV)
Difference in IgG-levels	T-test (VI) Wilcoxon rank test (VI) Chi Square test (binary data) (VI) Fisher's exact test (binary data) (VI)
Association between exposure and IgG-levels	Logistic regression (VI)
Differences in occurrence of symptoms and infections	Chi Square test (binary data) (VI) Fisher's exact test (binary data) (VI)

5 RESULTS

5.1 Concentrations and flora of airborne viable fungi

Airborne concentrations of viable fungi in indoor air environments are summarized in Table 8. Concentrations were significantly higher and the occurrence of fungal genera in the index buildings different from that in the reference buildings (I-III, V-VI). The most common fungal genera were *Penicillium* together with *Aspergillus, Cladosporium* and yeasts in a slightly different rank order depending on the study (I,III, V-VI). Some differences in the concentrations of individual genera were observed. Concentrations of A*spergillus* and *Oidiodendron* in the fall (I) and concentrations of A*spergillus* (I, III, V), *Penicillium* (I, III, V-VI), and yeasts (V-VI) in the winter were higher in the index than in the reference buildings. In study I, the mean ratio between an index and a matched reference building was 3 for the concentration of *Aspergillus* and 10 for *Penicillium*. In addition certain fungi, such as, *Acremonium* (I), ascomycetes (V), *Aspergillus versicolor* (III), *Botryosporium* (I) *Gliocephalis* (V), *Gliomastix* (V), *Gonatobotrys* (V), *Gonatorrhoidiella* (V), *Olpitrichum* (V), *Polyscytalum* (V), *Rhizopus* (V), *Stachybotrys* (I, V), *Tritirachium* (V), and *Ulocladium* (V), were detected in the index buildings in the winter, but not in the reference buildings. In addition, the following fungi occurred only in the damaged school (VI): *Aspergillus niger*, *Chrysosporium*, *Exophiala*, *Hyalodendron, Monocillium*, *Mucor*, *Paecilomyces*, *Rhizopus,* and *Scopulariopsis*. All fungal genera detected in indoor air of index and reference buildings are listed in Table 9. Altogether 47 and 34 fungal genera were found in the index and reference buildings, respectively.

The outdoor air concentrations of viable fungi were determined in the fall in study I and in the winter in study III. In the fall, the total concentrations of viable fungi as well as the concentrations of non-sporulating isolates, *Polyscytalum* and *Cladosporium* were higher in outdoor air than in indoor air (Table 8) (I). In winter, the geometric mean of the total concentrations of viable fungi was approximately 25 cfu/m^3 (III).

Table 8. The concentrations of viable fungi and the concentrations of the most common fungal genera or groups in index and reference residences and the corresponding samples of outdoor air in the fall. The results of studies III and VI are presented by combining the results obtained with MEA and DG18.

	Index		**Reference**		**Study**
	GM[a]	Range[b]	GM[a]	Range[b]	
Fall, indoor ($n_{ind/ref}$ =25/25)					
Total	250	19 - 7900	160	40 - 580	I
Penicillium	31	0 - 7900	16	0 - 72	I
Cladosporium	18	0 - 160	20	0 - 140	I
Aspergillus	4	0 - 76	2	0 - 20	I
Yeasts	14	0 - 74	16	0 - 200	I
Polyscytalum		0 - 40	3	0 - 38	I
Non-sporulating	54	0 - 1700	51	5 - 280	I
Fall, outdoor ($n_{ind/ref}$=9/9)					
Total	410	37 - 11000	190	37 - 630	I
Penicillium	14	0 - 95	12	0 - 76	I
Cladosporium	74	11 - 430	43	15 - 160	I
Aspergillus	1	0 - 11	2	0 - 15	I
Yeasts	23	0 - 790	10	0 - 83	I
Polyscytalum	16	5 - 93	3	0 - 54	I
Non-sporulating	190	19 - 9300	93	19 - 300	I
Winter, indoor ($n_{ind/ref}$=131/118)					
Total	120 - 363	7 - 54 000	58 - 61	11 - 1400	I, III, V-VI
Penicillium	38 - 90	0 - 8670	13 - 41	0 - 1400	I, III, V*-VI
Cladosporium	1 - 11	0 - 2370	1 - 5	0 - 71	I, III, V*-VI
Aspergillus	3 - 18	0 - 51500	1	0 - 187	I, III, V*-VI
Yeasts	5 - 14	0 - 403	1 - 8	0 - 62	I, III, V*-VI
Non-sporulating	7	0 - 269	2 - 9	0 - 83	I, V*-VI

[a] if more than one study, GM is presented as the range of GM's of different studies
[b] if more than one study, range represents the range of the different studies
* partly published in study V

Table 9. The prevalences (present in % of samples) of the most common fungal genera or species or groups detected less frequently (generally in <20% of samples) is also presented and denoted by an X. (Data from studies I, III, V, VI, partly published). The results are presented by combining the results obtained on MEA and DG18 (studies III, VI).

Fungal genera, species or group	Index (% of samples)	Reference (% of samples)
Penicillium	79 - 97	24 - 100
Aspergillus	53 - 73	19 - 55
Cladosporium	47 - 76	24 - 71
Yeasts	71 - 82	32 - 89
Non-sporulating isolates	36 - 71	12 - 76
Acremonium	X	X
Alternaria	X	X
Ascomycetes	X	
Aspergillus fumigatus	X	X
Aspergillus glaucus	X	
Aspergillus niger	X	
Aspergillus terreus	X	X
Aspergillus versicolor	X	X
Aureobasidium	X	X
Basidiomycetes	X	X
Botrytis	X	X
Chrysonilia		X
Chrysosporium	X	X
Chaetomium	X	
Eurotium	X	X
Exophiala	X	
Fusarium	X	X
Geotrichum	X	X
Geomyces	X	
Gliocephalis	X	
Gonatobotrys	X	
Gonatorrhoidiella	X	
Graphium		X
Humicola		X
Hyalodendron	X	X
Monocillium	X	X
Mucor	X	X
Oedocephalon	X	
Oidiodendron	X	X
Olpitrichum	X	X
Ovulariopsis	X	
Paecilomyces	X	X
Phialophora	X	X
Phoma	X	X
Polyscytalum	X	X
Rhizopus	X	
Rhinocladiella	X	X
Rhodotorula	X	
Scopulariopsis	X	X
Sphaeropsidales	X	X
Stachybotrys	X	
Trichoderma	X	X
Tritirachium	X	
Ulocladium	X	
Wallemia	X	X

The cumulative distributions of total concentrations of viable fungi in moisture-damaged and non-damaged residences in the combined study population are shown in Figure 1. (I, III, V and unpublished data). The results of study III are included as mean values of the several sampling periods.

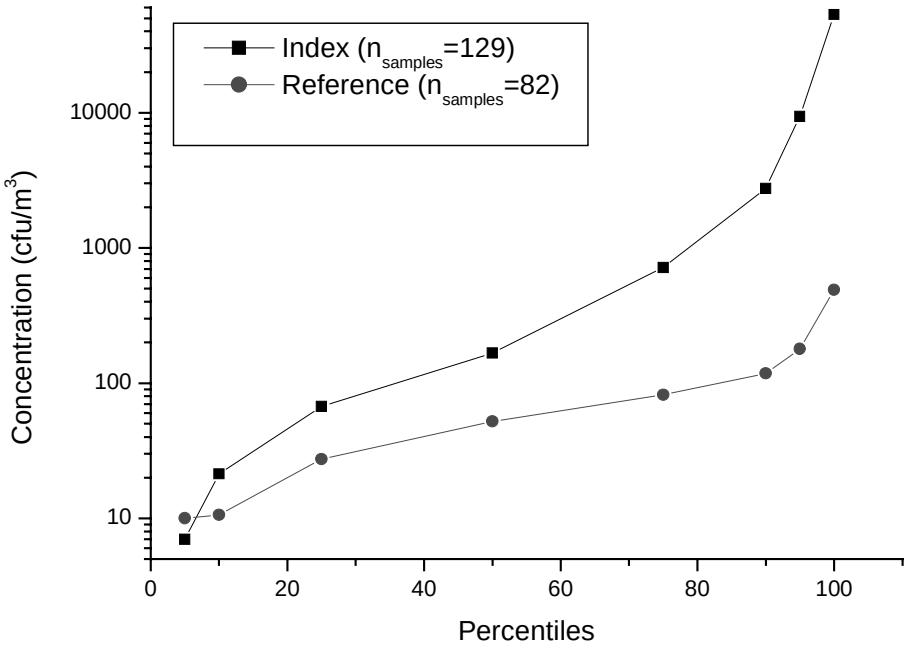

Figure 1. Cumulative distributions of concentrations of airborne viable fungi in index and reference residences.

In studies III and VI, the fungi were sampled on two growth media, MEA and DG18. The results obtained with MEA and DG18 correlated well in both studies for total concentration (r=0.92/074) and concentrations of *Penicillium* (r=0.91/0.53) and A*spergillus versicolor* (r=0.67/0.72). In study III, concentrations also correlated well for *Aspergillus* spp. (r=0.74). In the study VI, the correlation was moderate for yeasts (r=0.59), but poor (r=0.29) in the study III. Concentrations of *Cladosporium* correlated poorly in both studies (r=0.30/0.31).

5.2 Concentrations of airborne viable bacteria

In the winter, the concentrations of airborne viable mesophilic bacteria varied between 14 - 35 000 cfu/m^3 in the index residences and day-care centers and 79 - 18 000 cfu/m^3 in the corresponding reference buildings (II). In the index and reference schools, the respective ranges were 71 – 7 600 cfu/m^3 and <4 -2 100 cfu/m^3 (VI). In the fall, the range of the indoor concentrations of viable mesophilic bacteria was 36 - 4 600 cfu/m^3 in the index residences and day-care centers and 220 - 10 000 cfu/m^3 in the reference residences and day care centers (II). The differences between the index and reference buildings were not significant (II,VI).

Concentrations of airborne actinobacteria were <30 cfu/m^3 in all buildings. No differences between the index and reference buildings were found (II, VI). Actinobacteria were found in 26 % of samples taken from the index school and 6 % of the samples from reference schools. The total concentration of thermotolerant bacteria, being mainly actinobacteria, were <22 cfu/m^3 in both index and reference buildings. There were no differences in concentrations between indoor and outdoor air in the fall and no seasonal variation was detected (II).

5.3 Size distributions of viable fungi and bacteria

The highest concentrations of viable fungi and clearest differences between the index and reference buildings were usually in the size fraction of 2.1 –3.3 μm (II; Figure 2, III; Figure 2). Furthermore, there were differences in the size fraction >7μm in the fall (II; Figure 2) and in the size ranges 1.1-2.1μm (III; Figure 2) and in 3.3 –4.7 μm (II; Figure 2) in the winter. There were no differences in size distributions of airborne bacteria between the index and reference buildings either in the fall or in the winter samples (II).

The concentration difference ($p<0.05$) between the rooms of the index residence was seen in four size ranges (4.7-7μm, 3.3-4.7μm, 2.1-3.3 μm, 1.1-2.1 μm) (III; Figure 3A), while a difference between rooms was observed only in one size range (3.3-4.7μm) in the reference residence.

5.4 Temporal and spatial variation of concentrations of viable fungi

Concentrations of viable fungi varied significantly in different rooms of the index residence (III). The spatial variation was also seen in the concentrations of *Penicillium* (III).

In the dining room of the index residence, temporal variation was observed both within-day and within-season ($p \leq 0.001$). In the bedroom, both within-day and within-season differences were close to significant. All temporal differences in the reference residence were significant ($p < 0.001$). The total concentrations of fungi were usually higher in the morning than in the afternoon (III; Figure 4). This was true for both index and reference residences. The concentrations observed in November and in the beginning of December, especially in the reference residence, were mostly higher than those observed in February (III; Figure 4).

The variation in one room within one season was almost two orders of magnitude and even within same day the ratio of maximum and minimum values was 2-23 in the index residence and 2-9 in the reference residence. The difference between rooms within same day was up to 11 fold in the index residence, whereas in the reference residence it was less than 3 fold.

5.5 Proposal for sample size to characterize fungal level in residences during the winter months

Concentrations of viable fungi in indoor environments have temporal and spatial variation. Therefore, one short-time sample cannot characterize fungal contamination of a residence. The number of samples required for this purpose must be estimated. Sample size relates to the precision of measurement, yet the largest sample size is not necessarily the best sample size; cost and time must be considered. The margin of error, an indicator of the precision of the estimate, is defined as one-half the width of the confidence interval of the mean. The margin of error, (E), the significance level (α=0.05), and an estimate of the population standard deviation (σ) are combined to

estimate a sufficient and efficient sample size using the following equation: $n = (Z_{\alpha/2})^2 \sigma^2 / E^2$ (Freund and Wilson 1997), where is $Z_{\alpha/2} = 1.96$, when $\alpha=0.05$.

The margin of error was calculated using the log-transformed sample database of the index residence, E=0.785. The population standard deviation was obtained by dividing the population range by 4. The range between 5th and 95th percentile values of the merged database (n=129) of fungal concentrations were used to estimate the population standard deviation. The estimated value of σ was 1.32. The resulting sample size required to characterize the fungal concentration of an index residence was n=11.

5.6 Microbial concentrations and flora on surfaces

In studies V and VI, fungal concentrations varied between <1 – 360 000 cfu/cm^2 and <1 – 450 000 cfu/cm^2 on damaged surfaces in the index and reference buildings, respectively (V,VI). In the index residences (V), concentrations were above the limit of 1000 cfu/cm^2 in 51% of damaged surface samples, whereas the corresponding percentage was only 4 for the reference samples. In the index school (VI), concentrations of viable fungi were above 1000 cfu/cm^2 in four of 23 surface samples (17%), whereas all 14 samples taken from the reference school remained <200 cfu/cm^2. Bacterial concentrations varied between <1 – 12 400 cfu/cm^2 in both visibly damaged and undamaged surfaces in the index school, and between <1-450 000 cfu/cm^2 and <1 – 5 100 cfu/cm^2 in the reference school, respectively. Concentrations of actinobacteria varied from <1-820 cfu/cm^2 on damaged surfaces and <1 –180 cfu/cm^2 on undamaged surfaces in the index school, compared to the corresponding ranges of <1-5 cfu/cm^2 and <1-59 cfu/cm^2 in the reference school.

In study I, the rank order of frequency was rather similar to that in air samples in winter, except the genus *Aspergillus* which was rare in the surface samples, whereas in fall, there were several genera; *Acremonium, Oidiodendron, Geotrichum, Polyscytalum* and *Stachybotrys,* in air that were not found on surfaces.

Penicillium was the most frequently found genus on damaged surfaces (n=13) in the index school (54%) together with *Aspergillus versicolor* (38%), yeasts (31%), and *Acremonium* (31%). In the index residences (V), the rank order was different: yeasts were most commonly found (51%) followed by *Aspergillus* (26%), *Cladosporium* (26%) and *Penicillium* (17%). The fungal genera found on the surfaces were mostly the same as those in the indoor air (I, VI); only *Alternaria* (I), *Olpitrichum* (VI) and *Sphaeropsidales* (VI) were found on the surfaces but in none of the air samples taken from the corresponding building.

5.7 Building material samples

5.7.1 Microbial concentrations and flora in building material samples

Concentrations of viable fungi varied from <45 to $1.5x10^8$ cfu g^{-1} in visibly damaged materials (II,IV,V,VI). Significant differences ($p<0.05$) in fungal concentrations were observed between samples from different material groups (IV). Concentrations of fungi were higher in samples of wooden materials on both growth media compared to paints and glues, mineral insulation, ceramic products and plastics. In addition, concentrations were higher in paper than those from paints/glues or mineral insulation (IV).

Concentrations of viable bacteria varied from <45 to $6.5x10^7$ cfu g^{-1} in building materials (II,IV-VI). Significant differences ($p<0.05$) were observed in bacterial samples from different types of building materials (IV). Concentrations of bacteria were lower in mineral insulation than in wood, paper, ceramic products and plastics. No significant differences were found between bacterial concentrations in other building materials (IV). The correlation between total concentrations of viable fungi and bacteria was high ($R>0.6$) in all building materials except paper products.

The main genera observed in damaged building materials of the studied buildings were *Penicillium* (in 44-90% of samples) *(*II,V-VI), yeasts (35-41%) (II,V-VI), *Aspergillus* spp. (30-37%) (II,V)*, Cladosporium* (20%) (II), *Acremonium* (19-43%) (V-VI) and *Aspergillus versicolor* (39%) (VI). The fungal genera most commonly detected were mainly the same as in the indoor air (II,V-VI). When the mycoflora in indoor air and building materials of individual buildings were compared, 36-100% of the fungal genera found in

materials were also found in indoor air of the same building (II). Some fungal genera, i.e., *Ulocladium* and *Chaetophoma* (II), *Humicola*, *Monocillium*, *Ostracoderma* and *Staphylotichum* (V), and *Fusarium*, *Oidiodendron*, *Phialophora*, Sphaeropsidales group, *Stachybotrys* and *Tritirachium* (VI) were detected in the building material samples, but in none of the air samples of the corresponding building.

5.7.2 Occurrence of fungal genera and actinobacteria in various building materials

The occurrence of fungal genera, groups and actinobacteria in different building materials was studied from 1140 samples in study IV. The prevalences of 25 different fungal genera or groups (MEA) in six different damaged building material groups are shown in Figure 2. Figure 2 also shows the relative proportion that each genus or group contributed to the total fungal concentrations detected on MEA. The highest diversity of fungal genera was observed in wooden materials, where the number of genera or groups detected on MEA, N_{MEA} was 46, while the number on DG18, N_{DG18} was 39. On the mineral insulation, the numbers of detected genera were also high (N_{MEA}=41, N_{DG18}=36), while the diversity was lowest in gypsum boards (N_{MEA} = 18, N_{DG18}=18).

Penicillium was the most commonly occurring genus on MEA growth media in all building materials, and usually also the largest contributor to the total numbers of viable fungi. The prevalence of *Penicillium* in paper, wood and mineral insulation was significantly higher than that on plastics, ceramic products and paints. Yeasts were found most frequently in wooden materials. They contributed about 13-18% of the total fungal counts for most materials, except in ceramic products. *Acremonium* was found most frequently on ceramic products, in 35% of samples, and it contributed most to total fungal numbers in ceramic products and in paints and glues. *Aureobasidium* was found in 25% of samples from wood but in only 5-16% of samples of other materials. (Figure 2).

Aspergillus versicolor was most commonly observed on ceramic products, but also frequently in samples from paints and glues. Its contribution to total fungal concentrations was also highest on these materials. (Figure 2)

Cladosporium was found most commonly in paper material and mineral insulation, and the highest counts were usually found in paper products. *Stachybotrys* was commonly found in gypsum board (in 30% of samples), and at significantly higher frequencies than in any other material. *Stachybotrys* contributed 20% of total fungi in gypsum board, while its proportion in other materials was remarkably smaller (<4%). *Tritirachium* was observed most frequently in paints and glues (10%), but rarely in wood, mineral insulation, gypsum boards, and plastics. (Figure 2).

On DG18 media, the differences between materials were rather similar to those observed on MEA. *Penicillium* was again the most frequently found genus (37-67%). The high prevalence of yeasts in wooden materials was even emphasized on DG18: the difference was significant against all other materials except paper. While on MEA, *Cladosporium* was associated with paper and mineral insulation, it was isolated most commonly from mineral insulation on DG18.

Actinobacteria were found in 18-48% of different building materials. They were found most commonly on ceramic products (48%) and less frequently in paper, mineral insulation or gypsum board. Their average contributions to total bacterial concentrations were highest for paints and glues (22%), ceramic products (18%) and plastics (18%).

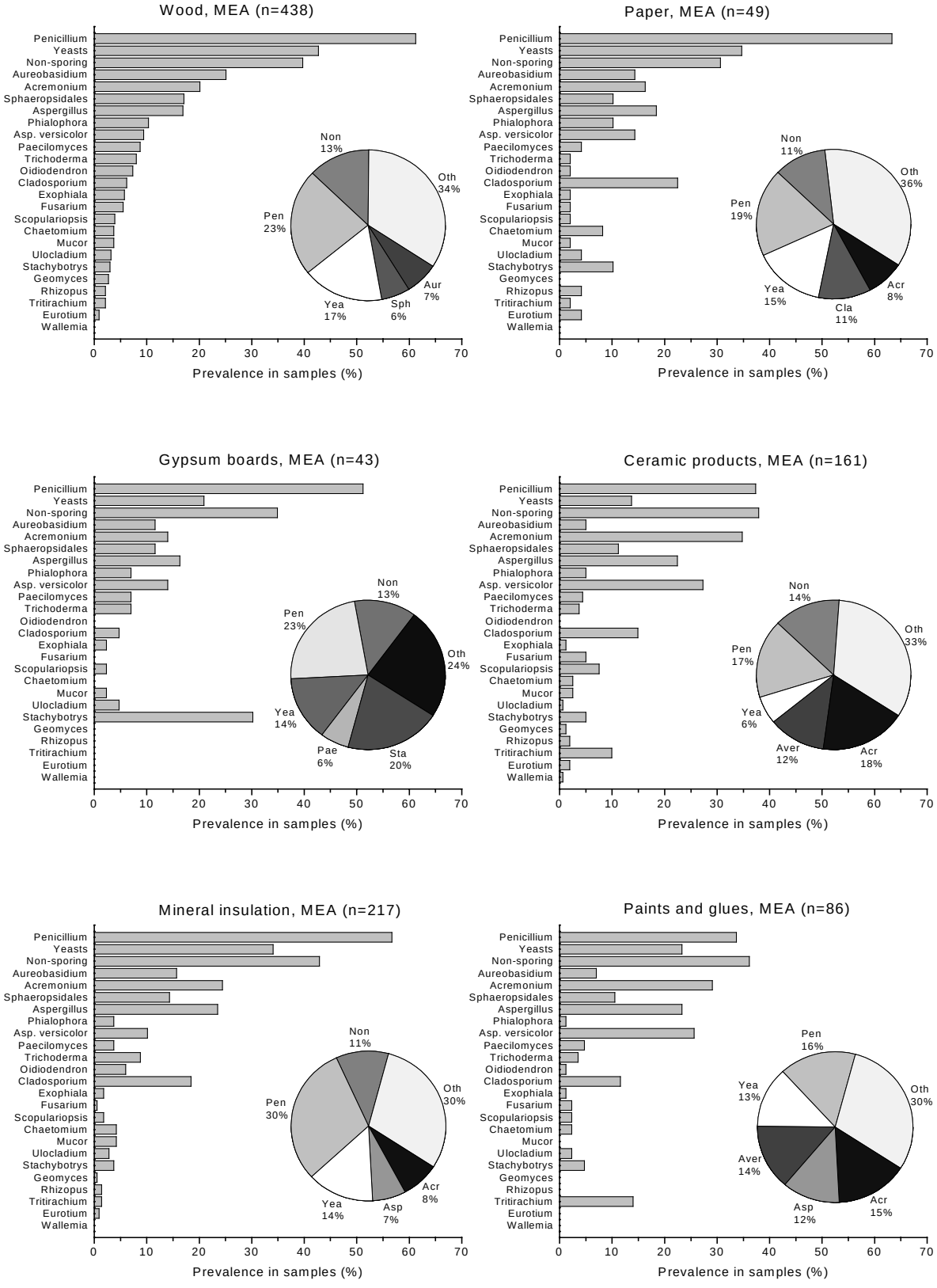

Figure 2. The prevalences of 25 different fungal genera or groups (MEA) in six different damaged building material groups. The figure also shows the relative proportion that each genus or group contributed to the total fungal concentrations detected on 2% MEA.

5.7.3 Concurrent occurrence of fungal genera and bacteria

Concurrent occurrences of two fungal genera or groups in different building materials at frequencies higher than those occurring spontaneously were tested using Chi square test, and are shown in study IV; Table 5. The most commonly observed genera or groups (*Penicillium, Aspergillus, Cladosporium* and yeasts), and the microbes that showed the most distinctive characteristics in relation to the different materials (*Acremonium*, *Aspergillus versicolor*, *Stachybotrys*, *Tritirachium, Phialophora,* Sphaeropsidales and actinobacteria) were included in the analyses.

Most of the concurrent occurrences of two fungal genera or microbial groups were associated with actinobacteria, *Penicillium, Acremonium, Aspergillus versicolor* and other *Aspergillus* spp. In all materials, actinobacteria and *Acremonium* were simultaneously found more often than expected. That was also the case for actinobacteria and *Aspergillus versicolor* in materials other than paper and gypsum board. In all materials except paper, the occurrence of Sphaeropsidales was associated with the presence of yeasts.

5.8 Occurrence of microbes in house dust samples

The concentrations of viable fungi in house dust are presented in study I; Table 4. In the fall, the concentrations of viable fungi were $7*10^3$-$1.2*10^6$ cfu/g and $1.7*10^4$-$4.2*10^5$ cfu/g in the index and reference buildings, respectively. The corresponding ranges were $3.8*10^4$-$6.8*10^5$ cfu/g and $3.0*10^4$-$4.0*10^5$ cfu/g in the winter. The difference between the buildings was not significant. The fungal genera were similar in both index and reference buildings. The most common genera were the same in the dust as in the air samples, but their order of frequency was different (Study I; Table 2). Yeasts and *Aureobasidium* were more common in the dust than in the air, while frequency of the non-sporulating isolates was lower in the dust than in the air samples.

5.9 Other environmental measurements

The concentrations of formaldehyde varied between <0.01 mg/m^3 and 0.14 mg/m^3 in both index and reference buildings and there was no difference between the groups (II). The mean concentrations of TVOC were 0.82 mg/m^3 in the index buildings and 0.62 mg/m^3 in the reference buildings, with no significant difference.

In study II, house dust samples were collected from beds and furniture. The samples were positive for dust mites in 25% of the index buildings and in 19% of the reference buildings. The difference was not significant.

5.10 Microbe-specific immunoglobulin G antibodies and symptoms and respiratory diseases

Microbe-specific immunoglobulin G antibodies

Positive IgG-findings were common among the occupants of both index and reference buildings (V-VI). In study V, higher antibody levels against most fungi were found in the study group than in the control group (Figures 3 and 4), whereas in study VI, mean IgG antibody levels seemed to be higher among the students from the reference school (Study VI). The difference, however, was significant only for *Cladosporium cladosporioides*, *Rhodotorula glutinis* and *Phoma macrostoma* ($p<0.05$).

In study VI, more children with elevated antibody levels (>75 percentile) came from the reference school than from the index school (Study VI; Table 1); the difference was significant for *Stachybotrys chartarum* and *Rhodotorula glutinis* ($p<0.05$). In contrast, 28 children in the index school had elevated IgG levels to *Penicillium notatum* and *Eurotium amstelodami* ($p=0.052$ vs. reference school). Among the grades 1-3, the number of children with elevated *Penicillium notatum* IgG levels was significantly higher in the index school than in the reference (Study VI; Figure 2). In grades 4-6, instead, the number of children with elevated IgG to *Rhodotorula glutinis*, *Cladosporium cladosporioides*, *Sporobolomyces salmonicolor, Tritirachium roseum* and *Streptomyces griseus* was higher in the reference school than in the index school (Study VI; Figure 2).

Compatibility between fungal flora found in each building and higher IgG-levels of its occupants to these microbes was seldom found in the index and reference buildings. In the reference group of the residences, high IgG-levels against *Trichoderma, Humicola, Stachybotrys* and *Rhizopus* were clustered to a few individuals who had no evident exposure in their homes (V). In Figures 3 and 4, the anti-*Cladosporium*- IgG and anti-*Phialophora*- IgG levels are shown as examples of IgG-levels in occupants' sera and the compatibility to the microbial findings (V). Figure 3 is an example of distribution of IgG levels of a commonly found microbe and figure 4 gives an example of a microbe found rarely.

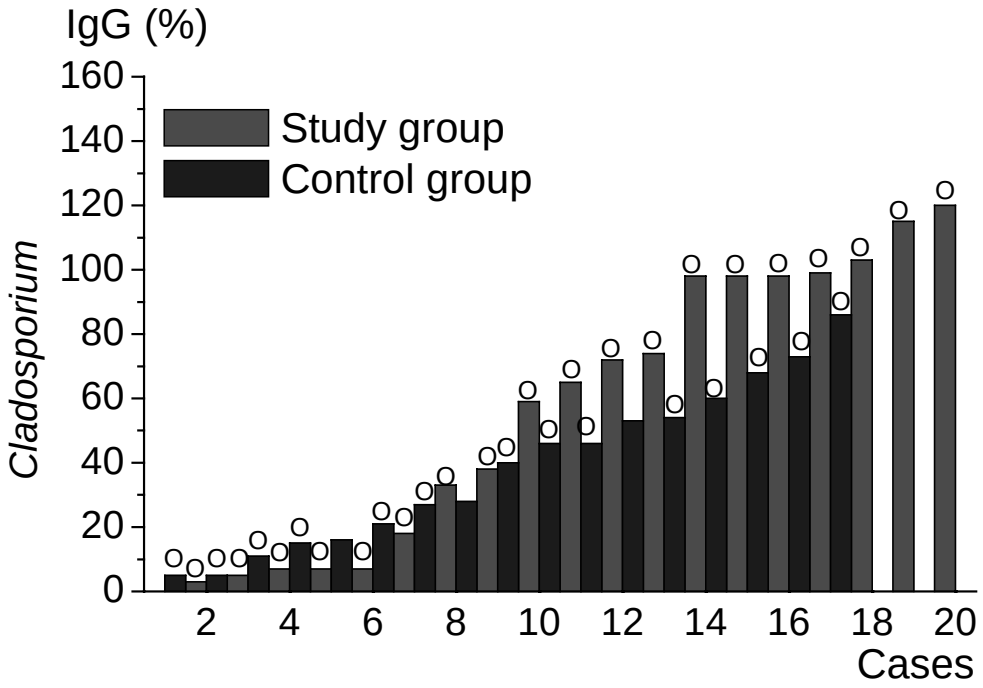

Figure 3. The IgG levels to *Cladosporium cladosporioides* in the sera of occupants in the index and reference residences. O indicates the presence of the genus in the environmental samples.

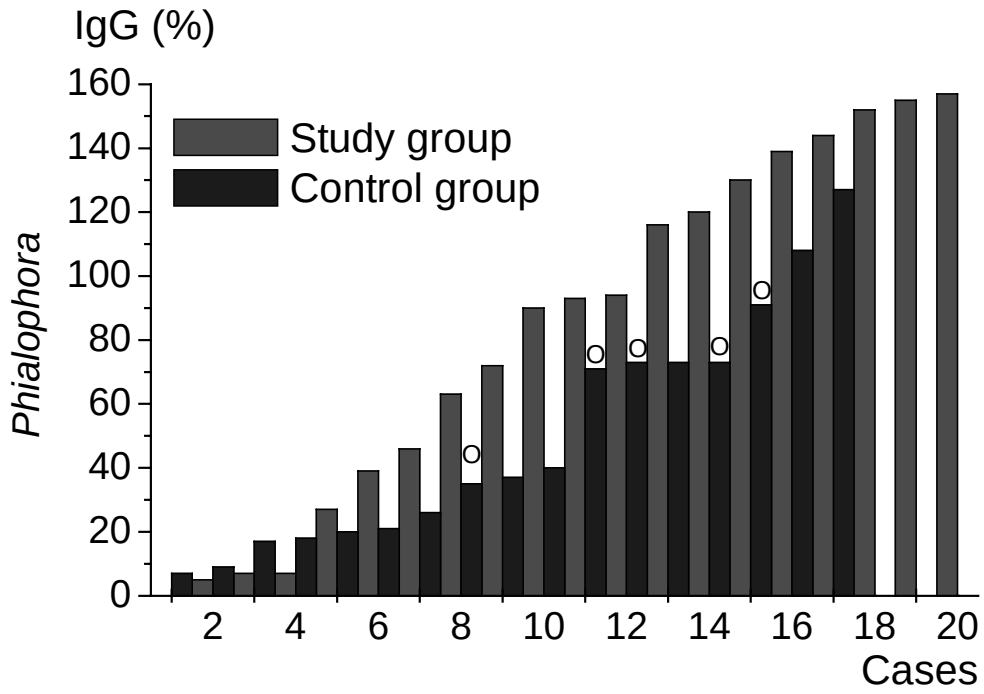

Figure 4. The IgG levels to *Phialophora bubakii* in the sera of the occupants of the index and reference residences. O indicates the presence of the genus in the environmental samples.

In study VI, associations between elevated serum IgG levels and microbial exposure were examined with crude rations of elevated IgG levels compared to microbial findings (Study VI: Table 1, Figure 1, Figure 2) and with logistic regression model. In the model, the associations between moisture damage in school, at home or at both places and mold specific IgG levels were analyzed. Age, gender, housing mode, having pets, passive smoking, asthma and atopy (allergic rhinitis, allergic conjunctivitis or atopic eczema) were included in the model. According to both of these analyses, moisture damage in the school was associated with elevated IgG levels to *Penicillium notatum* ($p<0.05$). Significant negative associations, however, were observed between elevated levels of *Stachybotrys chartarum* or *Rhodotorula glutinis* and moisture damage in the school ($p<0.05$). In addition, according to the model, elevated IgG levels to *Penicillium notatum* ($p<0.01$) and *Eurotium amstelodami (*$p<0.05$) were significantly associated with moisture damage both in the school and at home. Children with asthma had often

elevated IgG levels to *Geotrichum candidum* (OR=2.5, 95%CI=0.9-6.8) or to any of combined seven microbial strains (OR=2.3, 95%CI=0.9-6.3) (VI).

Symptoms and respiratory diseases

In study VI, the 212 children originally selected to the clinical study group had had either doctor diagnosed asthma, wheezing symptoms or prolonged cough during the previous 12 months. The prevalences of these health outcomes in the original population were 41% in the index school and 20% in the reference school ($p<0.001$) (Taskinen *et al.* 1999). The prevalence of asthma ($p<0.01$), allergic rhinitis ($p<0.05$) and conjunctivitis ($p<0.05$), doctor calls due to asthma ($p<0.05$) as well as parent reported wheezing attacks ($p<0.01$) were significantly higher in the reference school than in the index school. Reported allergic diseases were prevalent especially in the upper grades (4-6) of the reference school. However, the prevalence of night cough was significantly higher among the children of the index school compared to children attending the reference school ($p<0.05$).

The prevalences of respiratory symptoms (throat symptoms, hoarseness, dry cough, night cough, upcoming phlegm, nasal congestion, rhinitis, nose bleeding, eye irritation) are shown in Table 2 of study VI. In spring, dry cough and throat symptoms were more prevalent among children from the index school than among those from the reference school ($p<0.05$). The odds ratios for dry cough and throat symptoms in the index school were 2.91 (95% CI 1.11-7.90), and 2.99 (95% CI 1.14-8.11), respectively. The symptoms were more common in both schools in the fall. No significant differences were found in the occurrence of respiratory infections between the index group and the reference group.

6 DISCUSSION

Moisture and mold damage have been associated with several health effects (Verhoeff and Burge 1997, Peat *et al.* 1998), but the causal agents are still unknown. The aim of this work was to characterize the differences in indoor pollutants between moisture damaged (index) and non-damaged (reference) buildings. The buildings were classified as index and reference base on the building technical criteria. The differences were evaluated using two approaches: source characterization and exposure assessment. Levels of contaminants were examined in order to determine if there were abnormal indoor sources for microbes in indoors or the levels were considered as markers of exposure. The current studies concentrate on the airborne concentrations of viable microbes.

Samples for airborne microbes were mainly collected with a 6-stage impactor (Andersen 10-800). Its cut-off size for particle collection is 0.57 µm (Nevalainen *et al.* 1992) and aerodynamic size of microbial spores and cells vary in the size range 1-30 µm (Reponen *et al.* 2001). Therefore, most of the microbial particles will be collected. Collection of airborne microbes with 6-stage impactor supplemented with agar plates as a collection surface enables not only the identification of airborne microbes but also the determination of their size distribution. However, those microbial cells that are unable to form colonies on the selected medium or cells that are not viable will not be detected. It is known that viable counts comprise only about 1 % of the total counts of the spores in residential environments (Toivola *et al.* 2002). However, the methods to detect the total number concentration of microbial particles are poorly applicable to indoor environments such as homes, since the concentrations are near their detection limit (Toivola *et al.* 2002). The six-stage impactor is one of the recommended choices for collection of viable microbes proposed by the International Aerobiology Symposium and the American Conference of Governmental Industrial Hygienists (Jensen *et al.* 1992, Willeke and Macher 1999). In addition, differences between moisture-damaged and non-damaged buildings were characterized with the determination of viable microbes from building materials, surfaces and house dust.

Enumeration and description of microbial flora is dependent on culture media and the conditions used, and on interactions between the microbes present in the sample. Selection of culture media is a major factor in determining microbial growth, and should be taken into account when comparing results from different studies. In the current studies, microbial diversity in samples of air, materials, surfaces and house dust were mainly described for fungi and actinobacteria that could be detected on MEA or 2% MEA, DG18 and TYG. The combination of 2%MEA and DG18 is considered to encompass the majority of important indoor fungi (Samson *et al.* 1994, Samson *et al.* 1996) as MEA is compatible with more hydrophilic fungi and DG18 with xerophilic strains. However, cultivation on MEA favors fast growing *Penicillium* and *Aspergillus* at the expense of slower-growing species such as *Stachybotrys* (Samson *et al.* 1994, Andersen and Nissen 2000).

In addition to viable microbes, differences between the index and reference buildings were characterized by determination of house dust mites, TVOC, and formaldehyde, which are known to be associated with dampness (Konsgaard 1983, Reponen *et al.* 1991, Tucker 1991, Batterman 1995). Furthermore, the association between moisture and mold damage in building and symptoms or mold-specific IgG-levels were examined.

6.1 Concentrations and flora of airborne viable fungi

The concentrations of viable fungi varied between 10^1-10^4 cfu/m^3 and 10^1-10^3 cfu/m^3 in the index and reference buildings, respectively. In all studies, the concentrations of airborne viable fungi were higher in the index buildings than in the reference buildings. This is indicative of possible indoor air sources due to the existing moisture damage and was in concordance with studies in which mold damage has been associated with airborne fungal levels (Gallup *et al.* 1987, Hunter *et al.* 1988, Waegemakers *et al.* 1989, Verhoeff *et al.* 1992, Dharmage *et al.* 1999b, Johanning *et al.* 1999, Klánová 2000). Levels were <100 cfu/m^3 in about 38% of the index residences and in about 88% of the reference residences. Only about 27% and 1% of the levels were above >500 cfu/m^3 in the index and reference residences, respectively. This level of >500 cfu/m^3 is considered as “high” concentration indicating indoor air sources (Reponen *et al.* 1992,

Ministry of Social Affairs and Health 1997). Hence, the absolute levels of the viable fungi were high only in a few cases.

Penicillium with *Aspergillus* and *Cladosporium* were the most common indoor air fungal genera in all studies as observed in several other studies (Hunter *et al.* 1988, Miller *et al.* 1988, Waegemaekers *et al.* 1989, Strachan *et al.* 1990, Pasanen 1992, Pasanen *et al.* 1992c, Kuo and Li 1994, Li and Kuo 1994, Beguin and Nolard 1994, Dotterud 1995, Ren *et al.* 1999, Górny *et al.* 1999, Burge *et al.* 2000). Yeasts appeared commonly, also supporting the findings of Hunter *et al.* (1988), Pasanen (1992) and Pasanen *et al.* (1992c). In the current studies, the fungal composition of air samples was shown to be different in index and reference buildings. In wintertime sampling, concentrations of regularly found *Penicillium, Aspergillus* and yeasts were higher in the index than in the reference buildings. This finding has also been supported by some earlier studies showing higher levels of *Aspergillus, Cladosporium*, *Penicillium*, non-sporing fungi (including basidiomycetes), or yeasts in moldy homes (Strachan *et al.* 1990, Nevalainen *et al.* 1991, Pasanen 1992, Pasanen *et al.* 1992c, Garrett *et al.* 1998).

The observed differences in the fungal composition of air samples between index and reference residences during fall may be biased by outdoor air, since the same fungal genera were found in both indoor and outdoor air. Outdoor air is known to have a clear contribution to levels and flora of fungi in the indoor air (Fradkin *et al.* 1987, Reponen *et al.* 1992, Kuo and Li 1994, Li and Kuo 1994, DeKoster and Thorne 1995, Dharmage *et al.* 1999b, Burge *et al.* 2000, Su *et al.* 2001). In our climate with its snow cover on the ground in the winter, the contribution of outdoor air to indoor air levels is negligible (Reponen *et al.* 1992), which gives a good opportunity to examine the effect of indoor sources.

The fungal diversity in buildings was rather large; in all 47 and 34 fungal genera or groups were found in the index and reference buildings, respectively. The result was similar to that reported by Hunter *et al.* (1988) and Beguin and Nolard (1994). Sixteen fungal genera that were found in the air of the index buildings were never found in the air of the reference buildings indicating that moisture damage may change the fungal composition of the building. Part of these sixteen genera, such as *Rhizopus*, *Stachybotrys* and *Ulocladium,* were found sporadically, whereas genera such as

Gonatorrhoidiella and *Oedocephalon* were found only once. The recurrent findings of these uncommon genera may indicate moisture damage, thus supporting the earlier conclusion that certain microbes indicate moisture damage (Samson *et al.* 1994). On the other hand, *Chrysonilia, Graphium,* and *Humicola* were only found in the air of reference buildings showing the large diversity of fungal genera in air samples in general. The earlier results on the differences in the fungal composition between moisture damage and non-damaged buildings are rare and inconclusive. It has, however, been suggested by Flannigan (1997) that isolation of *Stachybotrys* from air should be interpreted differently from isolation of *Penicillium*, since e.g. spores of *Penicillium* and *Aspergillus* stay airborne longer than *Stachybotrys* due to their smaller particle size (Flannigan and Miller 1994). This could be assumed to be true for other rarely found genera, such as *Fusarium, Chaetomium* and *Ulocladium,* which are seldom airborne due their large spore size or way of producing spores (Samson *et al.* 1996).

The concept of the indicator microbe is complicated. For example, the identification to genus may not be accurate enough. On the other hand, according to Samson (1999) there is no general taxonomic consensus in the identification of some problematic genera such as *Penicillium*, *Aspergillus,* and *Fusarium*, which makes the use of indicator organisms even more problematic. In addition, certain microbes, such as *Aspergillus* species, actinobacteria and *Fusarium* are very common in agricultural environments (Kotimaa *et al.* 1984) which should be taken into account when investigating moisture damages in buildings connected with agricultural environments.

The results obtained with MEA and DG18 growth media correlated either well or moderately for total viable concentrations or concentrations of *Penicillium, Aspergillus* spp. and *Aspergillus versicolor*, but poorly for yeasts, especially for *Cladosporium,* which is partly supported by Ren *et al.* (2001). In addition, there were some genera that could be detected with the other medium only. For example *Stachybotrys* was rarely found on DG18 and *Wallemia* was rarely found on MEA. Usually higher levels and larger diversity were found with MEA as observed also by Ren *et al.* (2001). On the other hand, in the study of Wu *et al.* (2000a), the total fungal concentrations obtained with DG18 were higher than those with MEA and more genera were obtained with DG18. This study was done in a hospital with no evident moisture problems, which

might explain the findings supporting the use of DG18. When taking the cost effectiveness into consideration, the systematic use of two growth media, especially DG18, may not be justified.

6.2 Concentrations of airborne viable bacteria

The concentrations of airborne viable bacteria varied between 14 - 35 000 cfu/m^3 in the index buildings and 79 - 18 000 cfu/m^3 in the corresponding reference buildings. The observed range, especially in the index homes, is higher than that observed in earlier studies concerning homes (Nevalainen 1989, Nevalainen *et al.* 1991, Reponen *et al.* 1992, DeKoster and Thorne 1995, Rautiala *et al.* 1996, Ross *et al.* 2000). No differences, however, were found in the concentrations of airborne viable bacteria between the index and reference buildings. The bacterial concentrations were, however, occasionally over 4500 cfu/m^3, which according to guidelines of the Ministry of Social Affairs and Health of Finland (1997) is considered to be the upper normal level for total concentrations of airborne viable bacteria. Most of indoor air bacteria derive from humans and thus elevated concentrations of viable bacteria observed are probably attributable to overcrowding and inadequate ventilation (Nevalainen 1989, Otten and Burge 1999). In an earlier study by Nevalainen *et al.* (1991), abnormally high concentrations of airborne bacteria detected especially in moldy homes were also concluded to be the result of poor ventilation.

Mesophilic actinobacteria were determined from the bacterial samples, because in the earlier study of Nevalainen *et al.* (1991), their presence seemed to indicate moisture and microbial problems. However, in studies II and VI, the concentrations of mesophilic actinobacteria were <30 cfu/m^3 both in indoor and outdoor air and there were no differences in concentrations or occurrence of actinobacteria between the index and reference buildings. Thus, airborne actinobacteria did not associate with moisture damage in the buildings studied. However, there seem to be cases, where actinobacteria occur in high concentrations; in the study of Taskinen *et al.* (1997), some moisture damage buildings with high concentrations of airborne actinobacteria, 2700 cfu/m^3, have been observed. In addition, actinobacterial levels in the indoor air can increase up to 10^4 cfu/m^3 during the dismantling of moldy structures (Rautiala *et al.* 1996). The culturability of collected airborne actinobacterial spores varies extensively

and is affected by several factors, such as the species and sampling flow rate and hence an alternative to cultivation methods should to be developed (Reponen *et al.* 1998). In general, results concerning actinobacteria have been reported in only a few studies. In *in vitro* studies, they have been shown to be responsible for intense inflammatory and toxic responses (Hirvonen *et al.* 1997a and b) and hence, in future studies, their presence should be evaluated in more detail.

6.3 Size distributions of viable fungi and bacteria

There were differences between the index and reference buildings in the size distributions of viable fungi. The difference in the concentrations between the buildings was usually largest in the size fraction of 2.1 –3.3 µm, in which the highest increase in concentration due to demolition also occurred (Rautiala *et al.* 1996). These findings indicate significant differences in the exposure risk to people because particles of the 2-3 µm size range deposit effectively into the alveoli (Seinfeld 1986). Most fungal spores are only slightly hygroscopic and therefore their respiratory deposition is not significantly affected by changes in relative humidity (Reponen *et al.* 1996).

6.4 Temporal and spatial variation of concentrations of viable fungi

In the index residence, the concentrations of viable fungi were higher in the bedroom than in the dining room, whereas no such difference of practical value was observed in the reference residence. No or some spatial variation has been also observed earlier (Dotterud *et al.* 1995, Li and Kendrich 1995a, Ren *et al.* 1999, Ross *et al.* 2000, Ren *et al.* 2001). The spatial variation in the index residence was considered to be due to the more severe mold damage in the bedroom, because the activity level was considered to be similar in both residences. This is supported by studies observing higher levels in damaged buildings and basements with potential sources (Pasanen *et al.* 1992c, DeKoster and Thorne 1995, Ren *et al.* 1999). Concentrations in the other room of the index residence were significantly higher in four out of the six size fractions, whereas in the reference residence, that was the case only in one size range. These results of size distributions affirm the significant spatial variation in the index residence.

Total concentrations of viable fungi were usually higher in the morning than in the afternoon. This may reflect the activity peak in the mornings when adults went to work, school age children went to school and day care children first arrived and later went out to play. This is supported by studies, where highest concentrations have been seen during the highest level of activity (Flannigan 1992b) and due to resuspension of spores from a carpet by human activity (Buttner and Stetzenbach 1993). A trend towards higher concentrations at the beginning of the winter season was shown. One explanation for this might be the changes in the building because of the heating season. This suggestion, however, needs more research. Earlier studies have shown that fungal concentrations vary in time and space (Hunter *et al.* 1988, Verhoeff *et al.* 1990, Pasanen *et al.* 1992c). In study III, it was demonstrated that the variation is remarkable, even almost two orders of magnitude within one room within season and up to 11 fold between the rooms of the same residence.

6.5 Proposal for sample size to characterize fungal level in residences during the winter months

Due to the large variation of fungal concentration within space and time, a sampling campaign of 11 different days was shown to be needed to characterize the airborne fungal concentrations of a residence (III). Within-day variation should be addressed by sampling at different times in each of the 11 sampling days and within space variation by sampling two rooms of a subject residence during each sampling day.

Visible signs of mold in non-industrial environments are conclusive indicators of health risks (WHO 1990, Samson *et al.* 1994, Ministry of Social Affairs and Health 1997) and residences with such signs do not require air sampling - they require steps to control the problem. In residences where people are experiencing symptoms indicative of mold problems and where no visible signs of moisture or mold problems are seen, air sampling is justified to determine potential contamination. In the following section, the sampling campaign of 11 days is related to the pertinent guidelines in Finland (Ministry of Social Affairs and Health 1997). According to these guidelines, fungal concentrations above 100 cfu/m^3 and presence of indicator microbes (Samson *et al.* 1994) are alarm bells indicating a possible abnormal indoor source of fungi. The process should commence to identify the source and control it. Concentrations above 500 cfu/m^3 are

suggestive of an abnormal indoor source of fungi and action should be initiated to on identify and control the source. In study III, it was suggested that if neither of the above scenarios occurs, the sampling of indoor fungi must continue at least for 6 times over 2 months in the subject residence. If the coefficient of variation is less than 20 percent, no additional samples are needed; the mean fungal contamination of the subject residence is thought to be stable. If the coefficient of variation is larger than 20 percent, the remaining 5 samples should be taken over the next 2-month period to characterize fungal contamination of the residence. In occupational settings, it has been estimated that a reasonable approximation of an exposure distribution is often possible with about 10 measurements (AIHA 1998). Burge *et al.* (2000), however, have stated that microbial status of large buildings may not be sufficiently documented even with relatively extensive air sampling protocols. They observed no increase in fungal levels measured with 476 samples during 14 months even though fungal contamination was found in the air ducts of a large office building.

6.6 Microbial concentrations and flora on surfaces

Surface samples were taken to obtain more information about the fungal flora of the building and to show possible microbial growth on the surfaces. Surface samples are a kind of alternative for situations when building material samples cannot be taken. In the index residences, 17-51% of the samples taken from the surfaces with signs of moisture damage were above 1000 cfu/cm^2, which is considered to indicate microbial growth according to the guidelines of Ministry of Social Affairs and Health of Finland (1997). In the reference residences, the corresponding percentage was 0-4%. Thus, not all visibly damaged surfaces have active fungal growth, and on the other hand, there are local sites with active growth even in buildings with no evident moisture damage.

The fungal genera found in the surfaces were mostly the same as those in the indoor air, e.g. *Penicillium, Aspergillus, Cladosporium*, and yeasts. Only three genera, *Alternaria*, *Olpitrichum* and Sphaeropsidales, were found on the surfaces but in none of the air samples taken from the corresponding building. Thus, the surface samples gave only little additional information about the fungal flora in buildings as suggested by Tiffany *et al.* (2000), but confirmed the microbial findings of indoor air.

6.7 Building material samples

6.7.1 Microbial concentrations and flora in building material samples

Moist building materials provide the main substrates for microbial growth in buildings, and thus are one of the main contributing factors to intramural microbial emissions into indoor air. The concentration range of viable fungi in damaged building materials was large, <45 to $1.5x10^8$ cfu g^{-1} and similar to those reported earlier (Morey 1993, Andersson *et al.* 1997b, Carlson and Quraishi 1999, Etzel *et al.* 1998, Kujanpää *et al.* 1999, Johanning *et al.* 1999, Ellringer *et al.* 2000, Hodgson *et al.* 1998, Lappalainen *et al.* 2001). It was interesting, that even though the materials were identified as damaged from their appearance, microbial concentrations were often below the detection limit, e.g. in 12-35% of the samples depending on the material. Similar observations were reported by Kujanpää *et al.* (1999) and Reiman *et al.* (2000). These low concentrations may be partly due to an inability of fungi to grow on the selected growth media. Secondly, in some cases, fungal growth may not have developed if the material had dried quickly, although water damage had changed its visible appearance. It is also possible that spores had lost their ability to grow due to the drying of the material (Pasanen *et al.* 2000a). Using direct microscopy of the material, a close agreement between field and laboratory observations on fungal growth could be found, as suggested by Miller *et al.* (2000). Some genera, such as *Stachybotrys* may not be found with the cultivation from visibly damaged materials, even though it is microscopically found in these materials (Pasanen *et al.* 1992b Andersson *et al.* 1997b, Miller *et al.* 2000). Both these methods may be at least partly replaced with PCR-techniques, which have been already used for the detection and quantification of microbes in environmental samples and in experimental settings (Haugland *et al.* 1999, Buttner *et al.* 2001, Roe *et al.* 2001, Williams *et al.* 2001).

The fungal genera most commonly found in building materials were mainly the same as in the indoor air, even though the variety of fungal genera in indoor air was usually larger. A building material sample represents the flora of one microenvironment, a specific moist area in the building, but the flora of the indoor air is a result of several sources of fungi. Building material samples were, however, shown to give additional information of the composition of fungal flora in buildings, since some fungal genera

grew in the building materials, but were not seen in any of the corresponding air samples. This is supported by Rautiala *et al.* (1996), who detected fungal genera growing in the damaged materials in the air only during the dismantling of these materials. One reason for this phenomenon may be that some genera e.g. *Stachybotrys, Phialophora, Sphaeropsidales* and *Fusarium,* which produce their spores in slime (Samson *et al.* 1996), are not easily released from the growth. Secondly, genera with a larger spore size, for example *Stachybotrys, Ulocladium* and *Chaetophoma,* do not stay airborne for long periods of time as do the typical indoor air fungi which have a smaller spore size, such as *Penicillium* and *Aspergillus (*Samson *et al.* 1996).

6.7.2 Microbial concentrations and diversity in various building materials

The highest median concentrations of fungi were observed in wooden and paper materials and lowest in mineral insulation, ceramic products, and paints and glues. Thus, wood and paper seem to offer more favorable conditions for fungal growth than do other common building materials, such as gypsum board, mineral insulation, ceramics, plastics, and paints. However, the study confirmed that even materials which are not readily biodegradable, such as mineral-based insulation or ceramics, may provide conditions for fungal growth.

Fungal and bacterial numbers correlated well in the samples, except in paper materials, meaning that a sample with a high fungal count had usually also a high bacterial count. The range of bacterial concentration was wide, 10^0-10^8 cfu g^{-1}. Mineral insulation had significantly lower bacterial concentrations than other materials.

The diversity of fungal genera in different materials varied greatly. The highest diversity was observed in wooden materials (number of various genera 46) and mineral insulation (41), while lowest in gypsum board (18). The variation in diversity may be due, in part, to the larger number of samples from wooden building materials and mineral insulation compared to samples from gypsum board, which may increase the probability of observing more genera. On the other hand, wooden materials provide relatively good nutrient conditions for growth. Mineral insulation is not a nutrient-rich material but may act like a filter for outdoor air microbes, which may partly explain the

variety of fungi found in these samples. Plastics contain few nutrients, but were associated with a large number of genera, which may indicate the utilization of accumulated dust on plastic materials as a nutrient source for growth.

Penicillium was the most common genus in moisture damaged building materials and comprised the largest fraction of total viable fungal concentrations, in agreement with earlier studies (Andersson *et al.* 1997b, Gravesen *et al.* 1999, Tuomi *et al.* 2000, Reiman *et al.* 2000). The high prevalence of *Penicillia* was to be expected, being primary colonizers, fast growing, and not very demanding for nutrients and moisture (Grant *et al.* 1989) and even tolerant to fluctuating or dry conditions (Adan 1994, Korpi *et al.* 1998). *Penicillium* also appeared most frequently concurrently with other genera. The second most common fungal group were yeasts, appearing most frequently in wooden materials, and comprising a high proportion of total viable fungal concentration in most materials.

One of the most distinctive findings in the material study was the high prevalence of *Stachybotrys* in gypsum board (in 30% of samples). This fungus has been often linked to this material (Andersson *et al.* 1997b, Gravesen *et al.* 1999, Hung 1999). It is noteworthy, however, that in 70% of gypsum board samples no *Stachybotrys* was found, but growth was dominated by other genera. The occurrence of *Stachybotrys* in gypsum board was not associated with simultaneous occurrence of any other genus. *Stachybotrys* was also observed frequently in paper materials, but less often than in gypsum board even though it is known to be highly cellulolytic (Gravesen *et al.* 1994).

Ceramic products and paints and glues seemed to favor the growth of *Aspergillus versicolor* and *Acremonium*. These microbes were frequently found concurrently not only in these materials, but also in wood. *Tritirachium* also was associated with these fungi and materials, even though its proportion of total fungal concentrations was low. The observation of Ezeonu *et al.* (1994) that *Aspergillus versicolor* was the most common colonizer in samples of new fiberglass was not supported.

Actinobacteria were found commonly (18-48%) in building materials, with an equal frequency as yeasts. Actinobacteria were found especially on ceramic products, which may be due to their capability to tolerate alkaline conditions (Suutari *et al.* 2000).

Interestingly, although commonly occurring and having both inflammatory and toxic potential (Hirvonen *et al.* 1997a and b, Andersson *et al.* 1998), actinobacteria have been rarely discussed in studies concerning building related microbes.

Actinobacteria and *Acremonium* were found concurrently in all materials, and actinobacteria and *Aspergillus versicolor* in all materials other than paper and gypsum board. Members of the Sphaeropsidales were strongly associated with the occurrence of yeasts in most building materials. These are, to our knowledge, new findings. Due to the consistency of the finding, it deserves more attention in future studies.

6.8 Occurrence of fungi in house dust samples

Concentrations of viable fungi in house dust varied $7*10^3$-$1.2*10^6$ cfu/g in the index buildings and $1.7*10^4$-$4.2*10^5$ cfu/g in the reference buildings, having no significant difference. The levels were close to those reported by Miller *et al.* (1988) and Verhoeff *et al.* (1994a). The fungal genera were similar in both index and reference buildings. It seems that with sampling of house dust from the vacuum cleaner and subsequent microbial analyses, a building with moisture damage cannot be distinguished from a normal building. This is supported by Verhoeff *et al.* (1994b), Ren *et al.* (1999) suggesting that dust samples do not reflect the exposure. The reason for similarity in concentrations and composition of fungal flora in house dust of the index and reference buildings may be that the majority of the viable fungi in the dust originate from outdoors and from brief concentration peaks from normal sources (Lehtonen *et al.* 1993).

6.9 Other environmental measurements

In order to identify differences between the index and reference buildings several other methods were also screened (II). The studied buildings included six moisture damaged residences and three moisture damaged day-care centers and their matched controls (see also section 4.1). The methods were determinations of formaldehyde, TVOC and occurrence of house dust mites. The concentrations of formaldehyde were <0.15 mg/m^3 being lower than the guideline values in Finland: 0.15 mg/m^3 for the buildings built after 1983 and 0.30 mg/m^3 for older buildings (Ministry of Social Affairs and Health 1997). Thus, the formaldehyde concentrations did not appear to be elevated as a result of

moisture and mould problems, nor did they explain the differences in symptoms of the occupants in the index and reference buildings.

The means of TVOC concentrations were 0.82 mg/m^3 and 0.62 mg/m^3 in the index and in the reference buildings, respectively, with no significant difference. The concentrations were low compared to a study showing an association between sick building syndrome and VOC (Wallace *et al.* 1990), but agreed with the calculated mean values (weighted average geometric mean = WAGM) 1.13 mg/m^3 and 0.52 mg/m^3 for established and complaint dwellings, respectively (Brown *et al.* 1994). The concentrations in study II, even in the reference buildings, were higher than the average concentrations reported earlier in smoking homes and in non-smoking homes (Hoskins *et al.* 1993) or in 50 non-problem houses in Finland (Kostiainen 1995). Thus, the importance of TVOC measurements in detecting a moisture or mold problem must be questioned.

House dust mites (HDM) occurred in bed dust to a similar degree, about 20% in both buildings with moisture problems and in the reference buildings. This is a low prevalence compared to the results of O'Rourke *et al.* (1993) and Flannigan *et al.* (1993) with a 50% of prevalence and van Strien *et al.* (1994) and Leung *et al.* (1998) with a 100% prevalence. The low prevalence may be partly due to Scandinavian conditions, where indoor air relative humidity is <50% for most of the time, which is the critical humidity level for the survival of HDM. The infrequent occurrence of mites in the northern climate was also shown by Raunio *et al.* (1998), who did not detect the major allergen of house dust mite *Dermatophagoides pteronyssinus* (*Der p 1*) in any of the house dust samples collected from floor carpeting in 30 residences. Hence, mites seem to be generally ubiquitous and their occurrence is rather associated with favorable microclimatic conditions than with moisture problems in a house.

6.10 Microbe-specific immunoglobulin G antibodies and respiratory symptoms and diseases

Microbe-specific immunoglobulin G antibodies

Higher mold-specific IgG-levels were found among residents of moldy buildings than in residents of control homes. However, only two IgG findings supported the hypothesis that indoor exposure to fungi in school increases mold-specific IgG levels. The higher percentages of elevated IgG levels in the index school to *Penicillium notatum* and *Eurotium amstelodami* were observed. This was supported by the multivariate analysis, since the elevated IgG levels to *Penicillium notatum* were associated with moisture damage in school alone and in both school and home and high levels of *Eurotium amstelodami* were associated with the presence of moisture damage in both school and home. These microbes were more abundant in the index school; *Penicillium* in significantly higher levels and *Eurotium* more frequently than in the reference school. This is in line with preliminary findings in adults by Makkonen *et al.* (2001). Moisture damage in the school was negatively associated with elevated IgG levels of *Stachybotrys chartarum* and *Rhodotorula glutinis* with both univariate and multivariate analysis adjusted for potential confounding factors. A cluster of high IgG-levels against *Trichoderma, Humicola, Stachybotrys* and *Rhizopus* were found from a few persons with no evident exposure in their homes. Evidently, mold-specific serum IgG determinations do not reflect sensitively enough an exposure in the moisture and mold damaged buildings either in the school or home environment.

Symptoms and respiratory diseases

Cough and throat symptoms among schoolchildren were associated with mold problems in the school. Of special importance is the association between mold exposure and night cough, which often precedes the development of asthma (Remes *et al.* 1998). The association of the respiratory symptoms with the indoor exposure to building-related moisture and mold is consistent with previous studies (Dales *et al.* 1991, Spengler *et al.* 1994, Koskinen *et al.* 1997). In general, there was a trend towards more prevalent irritation symptoms in the index school than in the reference

school. This trend was present in spite of the fact that in this cohort, asthma and allergy were more prevalent in the reference school.

7 CONCLUSIONS

The moisture-damaged buildings were characterized and compared to the reference buildings with several methods including microbial sampling of air, surfaces, building materials and dust, determination of volatile organic compounds, formaldehyde and house dust mites. In addition, mold-specific serum IgG-levels of occupants in moisture-damaged and reference buildings were assessed. The following conclusions can be drawn from the results:

1. The wintertime concentrations of total viable fungi (10^1-$5*10^4$ cfu/m^3) and concentrations of *Penicillium* (10^0-10^4 cfu/m^3), *Aspergillus* (10^0-10^4 cfu/m^3), and yeasts (10^0-10^2 cfu/m^3) in the moisture damaged buildings were higher than in the reference buildings. Higher levels of fungi were observed especially in the particle size fraction of 2-3 µm. In addition, the fungal diversity was larger in the moisture damaged buildings. Certain fungal genera, such as *Stachybotrys, Ulocladium, Tritirachium* and *Exophiala*, were detected only in the air of the moisture problem buildings. No differences were observed in the concentrations or occurrence of the other parameters: airborne viable bacteria, TVOC, formaldehyde, fungi in house dust and house dust mites.

2. The fungal concentrations in moisture damaged and reference buildings overlapped in most cases in moisture damaged and reference buildings, and hence no absolute level can be said to typically indicate the existence of moisture damage. However, by examining both the levels and flora of the air samples, indications of moisture problems can be achieved. The determination of microbial levels and flora especially in building materials, but also on surfaces, were shown to give additional information on the microbial flora in building and this knowledge can be utilized in source characterization.

3. The temporal variation of the fungal concentrations was significant both in index and reference residences, whereas spatial variation affected mostly the levels in the index residence. In order to reliably ascertain the fungal level of a residence, a sampling campaign of 11 different sampling days in two rooms was proposed. In

cases where there is only minor variation between six measurement days (coefficient of variation <20%), no additional sampling is needed.

4. Fungal diversity in moisture damaged building materials was large. Fungal growth was associated with bacterial growth. *Stachybotrys* was associated with gypsum boards. A*cremonium, Aspergillus versicolor* and actinobacteria were associated with ceramic materials and they occurred often together on the other materials as well. Sphaeropsidales and yeasts occurred often concurrently in damaged building materials.

5. Mold-specific serum IgG levels were associated with only a few microbial findings. The occurrence of elevated serum levels was contradictory in exposed and non-exposed population in different studies. It can be concluded that mold-specific serum IgG levels are not sensitive enough to indicate the current exposure in a moisture damaged home or school environment.

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